APPLICATION

FOR

UNITED STATES LETTERS PATENT

TITLE: IMMEDIATE EARLY GENES AND METHODS OF USE THEREFOR

APPLICANT: PAUL F. WORLEY AND ANTHONY LANAHAN

"EXPRESS MAIL" Mailing Label Number EL253771810US
Date of Deposit February 5, 1999
I hereby certify under 37 CFR 1.10 that this correspondence is being deposited with the United States Postal Service as **"Express Mail Post Office To Addressee"** with sufficient postage on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

M.E. Augustine

M.E. Augustine

Attorney Docket 10496/005001

IMMEDIATE EARLY GENES AND METHODS OF USE THEREFOR

Related Applications

This application claims priority to U.S. provisional application nos. 60/074,518, filed February 12, 1998 and 60/074,135, filed February 6, 1998, both of which are incorporated herein by reference.

Statement as to Federally Sponsored Research

Funding for the work described herein was provided by the federal government, which may have certain rights in the invention.

BACKGROUND

1. Technical Field

The present invention generally relates to gene expression and more specifically to immediate early genes in the brain and polypeptides encoded by such immediate early genes.

2. Background Information

An immediate early gene (IEG) is a gene whose expression is rapidly increased immediately following a stimulus. For example, genes expressed by neurons that exhibit a rapid increase in expression immediately following neuronal stimulation are neuronal IEGs. Such neuronal IEGs have been found to encode a wide variety of polypeptides including transcription factors, cytoskeletal polypeptides, growth factors, and metabolic enzymes as well as polypeptides involved in signal transduction. The identification of neuronal IEGs and the polypeptides they encode provides important information about the function of neurons in, for example, learning, memory, synaptic transmission, tolerance, and neuronal plasticity.

SUMMARY

The present invention involves methods and materials related to IEGs. Specifically, the invention provides isolated IEG nucleic acid sequences, cells that contain isolated IEG nucleic acid, substantially pure polypeptides encoded by IEG nucleic acid, and antibodies having specific binding affinity for a polypeptide encoded by IEG nucleic acid. In addition, the invention provides cDNA libraries enriched for IEG cDNAs, isolated nucleic acid derived from such cDNA libraries, and methods for treating conditions related to a deficiency in a neuron's IEG responsiveness to a stimulus.

In one aspect, the invention features an isolated nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. The isolated nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60. The hybridization conditions can be moderately or highly stringent hybridization conditions.

In another embodiment, the invention features an isolated nucleic acid having a nucleic acid sequence that encodes an amino acid sequence at least five amino acids in length. The amino acid sequence contains at least three different amino acid residues, and is identical to a contiguous portion of sequence set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, or 62.

Another embodiment of the invention features an isolated nucleic acid having a nucleic acid sequence at least 60 percent identical to the sequence set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60.

Another embodiment of the invention features an isolated nucleic acid having a nucleic acid sequence that encodes an amino acid sequence at least 60 percent identical to the sequence set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, or 62.

Another embodiment of the invention features an isolated nucleic acid having a nucleic acid sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60.

In another aspect, the invention features a substantially pure polypeptide having an amino acid sequence encoded by a nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. The nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60.

In another embodiment, the invention features a substantially pure polypeptide having an amino acid sequence as set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, or 62.

Another embodiment of the invention features a substantially pure polypeptide having an amino acid sequence at least 60 percent identical to the sequence set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, or 62.

Another embodiment of the invention features a substantially pure polypeptide having an amino acid sequence at least five amino acids in length. The amino acid sequence contains at least three different amino acid residues, and is identical to a contiguous stretch of sequence set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, or 62.

Another aspect of the invention features a host cell (e.g., a eukaryotic or prokaryotic cell) containing an isolated nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. The isolated nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60.

Another aspect of the invention features an antibody (e.g., a monoclonal or polyclonal antibody) having specific binding affinity for an amino acid sequence encoded by a nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. The nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60.

Another aspect of the invention features a cDNA library having a plurality of clones with each clone having a cDNA insert. In addition, at least about 15 percent (e.g., at least about 20 or 25 percent) of the clones have cDNA derived from immediate early genes (e.g., immediate early genes responsive to a maximal electroconvulsive seizure). The cDNA library can be a subtracted cDNA library. For example, the subtracted cDNA library can be the IEG-Reg or IEG-Lg cDNA library.

Another aspect of the invention features an isolated nucleic acid derived from a cDNA library. The cDNA library has a plurality of clones with each clone having a cDNA insert. In addition, at least about 15 percent of the clones have cDNA derived from immediate early genes. The isolated nucleic acid can have a nucleic acid sequence of an immediate early gene.

Another aspect of the invention features a method of obtaining immediate early gene nucleic acid. The method includes providing a cDNA library having a plurality of clones with each clone having a cDNA insert. In addition, at least about 15 percent of the clones have cDNA derived from immediate early genes. The method also includes contacting at least a portion of the cDNA library with a probe containing at least one nucleic acid having a nucleic acid sequence derived from an immediate early gene, and selecting a member of the plurality of clones based on the hybridization of the at least one nucleic acid to the member under hybridization conditions.

Another aspect of the invention features a method of treating an animal (e.g., human) having a deficiency in a neuron's immediate early gene responsiveness to a stimulus. The

method includes administering a nucleic acid to the animal such that the effect of the deficiency is minimized. The nucleic acid has at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. In addition, the nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60. The deficiency can include a reduced level of expression of an immediate early gene. In addition, the stimulus can influence learning or memory. For example, the stimulus can include a maximal electroconvulsive seizure.

In another embodiment, the invention features a method of treating an animal (e.g., human) having a deficiency in a neuron's immediate early gene responsiveness to a stimulus. The method includes administering a therapeutically effective amount of a substantially pure polypeptide to the animal such that the effect of the deficiency is minimized. The polypeptide contains an amino acid sequence encoded by a nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. The nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60.

Another embodiment of the invention features a method of treating an animal (e.g., human) having a deficiency in a neuron's immediate early gene responsiveness to a stimulus. The method includes administering an effective amount of cells to the animal such that the effect of the deficiency is minimized. The cells contain a nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. In addition, the nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a

sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60.

Another embodiment of the invention features a method of treating an animal (e.g., human) having a deficiency in a neuron's immediate early gene responsiveness to a stimulus. The method includes administering a therapeutically effective of antibodies to the animal such that the effect of the deficiency is minimized. The antibodies have specific binding affinity for an amino acid sequence encoded by a nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. The nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60. The deficiency can include an elevated level of expression of an immediate early gene.

Another aspect of the invention features a method of identifying a compound that modulates immediate early gene expression. The method includes contacting a test compound with an immediate early gene nucleic acid, and determining whether the test compound effects the expression of the immediate early gene nucleic acid. The presence of an effect indicates that the test compound is a compound that modulates immediate early gene expression. The immediate early gene nucleic acid can contain a nucleic acid sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60. The effect can be a reduction or increase in the expression of the immediate early gene nucleic acid.

In another embodiment, the invention features a method of identifying a compound that modulates immediate early gene polypeptide activity. The method includes contacting a test compound with an immediate early gene polypeptide, and determining whether the test compound effects the activity of the immediate early gene polypeptide. The presence of an effect indicates that the test compound is a compound that modulates immediate early gene

polypeptide activity. The immediate early gene polypeptide can contain an amino acid sequence encoded by a nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. The nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60. Alternatively, the immediate early gene polypeptide can contain an amino acid sequence as set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, or 62. The effect can be a reduction or increase in the activity of the immediate early gene polypeptide.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

DETAILED DESCRIPTION

The present invention provides methods and materials related to IEGs. Specifically, the invention provides isolated IEG nucleic acid, cells that contain isolated IEG nucleic acid, substantially pure polypeptides encoded by IEG nucleic acid, and antibodies having specific binding affinity for a polypeptide encoded by IEG nucleic acid. In addition, the invention provides cDNA libraries enriched for IEG cDNAs, isolated nucleic acid derived from such

cDNA libraries, and methods for treating conditions related to a deficiency in a neuron's IEG responsiveness to a stimulus.

The present invention is based on the discovery of nucleic acid clones for many different neuronal IEGs. Specifically, nucleic acid clones for different neuronal IEGs were isolated and identified based on the ability of each IEG to rapidly increase expression upon seizure induction by a maximal electroconvulsive seizure (MECS) method (Cole *et al.*, *J. Neurochem.* 55:1920-1927 (1990)). It is important to note that MECS induction can be considered a model to study long-term plasticity relevant to learning and memory since it is known that a single MECS can produce extremely robust and long lived potentiation of synaptic contacts in the hippocampus and block spatial learning (Barnes *et al.*, *J. Neurosci.* 14:5793-5806 (1994)). Thus, MECS-responsive IEGs can influence neuronal activities involved in brain functions such as learning and memory. Moreover, the isolation and identification of IEG nucleic acid not only provides research scientists with information about neuronal activity and gene regulation but also provides methods and materials that can be used to manipulate brain function.

Each isolated IEG nucleic acid described herein can be used to produce a polypeptide. In addition, each IEG nucleic acid can be used to identify cells that are responsive to MECS induction. For example, an IEG nucleic acid can be labeled and used as a probe for *in situ* hybridization analysis. Clearly, having the ability to identify MECS-responsive cells provides one with the ability to isolate or monitor specific brain regions that are involved in learning. Further, any of the isolated partial IEG nucleic acid sequences can be used to obtain a full-length clone that encodes an IEG polypeptide. For example, a fragment from an isolated IEG nucleic acid can be radioactively labeled and used to screen a library such that a full-length clone is obtained.

Cells containing isolated IEG nucleic acid can be used to maintain or propagate the isolated IEG nucleic acid. In addition, such cells can be used to produce large quantities of polypeptides that are encoded by isolated IEG nucleic acid. Further, cells containing isolated IEG nucleic acid can be used to generate virus particles containing the isolated IEG nucleic acid. Such virus particles can be used *in vitro* or *in vivo* to provide other cells with the isolated IEG

nucleic acid. The polypeptides encoded by IEG nucleic acid can be used as immunogens to produce antibodies. Such antibodies can be used to identify MECS-responsive cells, monitor the level of polypeptide expression following MECS induction, and isolate polypeptides directly from animal tissue.

cDNA libraries enriched for IEG cDNAs can be used to isolate novel IEG cDNA. Clearly, the isolation of novel IEG cDNAs is important to further the understanding of brain function. In addition, isolated nucleic acid derived from such cDNA libraries can be used to produce polypeptides as well as identify cells that are responsive to a stimulus such as MECS induction.

It is important to note that isolated IEG nucleic acid, cells containing isolated IEG nucleic acid, substantially pure IEG polypeptides, and anti-IEG polypeptide antibodies can be used to treat conditions associated with a deficiency in a neuron's ability to express IEGs in response to a stimulus such as MECS. A condition associated with a deficiency in a neuron's IEG responsiveness to a stimulus is any physiological condition characterized as having a lack of a normal level of responsiveness. For example, when a deficiency in a neuron's responsiveness to MECS is characterized as a non- or under-expression of a particular IEG polypeptide by that neuron, the organism having the condition can be treated with isolated IEG nucleic acid, cells containing isolated IEG nucleic acid, or substantially pure IEG polypeptides such that the effect of the deficiency is minimized. Alternatively, when a deficiency in a neuron's responsiveness to MECS is characterized as an over-expression of a particular IEG polypeptide by that neuron, the organism having the condition can be treated with anti-IEG polypeptide antibodies or the anti-sense strand of an isolated IEG nucleic acid such that the effect of the deficiency is minimized.

In addition, isolated IEG nucleic acid, cells containing isolated IEG nucleic acid, substantially pure IEG polypeptides, and anti-IEG polypeptide antibodies can be used to identify pharmaceutical compounds that can be used to treat diseases such as epilepsy, age-dependent memory decline, stroke, and drug addiction. For example, a compound that modulates IEG nucleic acid expression or IEG polypeptide activity can be identified by contacting a test

compound with either the IEG nucleic acid or polypeptide, and determining whether the test compound effects expression or activity.

The term "nucleic acid" as used herein encompasses both RNA and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. The nucleic acid can be double-stranded or single-stranded. Where single-stranded, the nucleic acid can be the sense strand or the antisense strand. In addition, nucleic acid can be circular or linear.

The term "isolated" as used herein with reference to nucleic acid refers to a naturally-occurring nucleic acid that is not immediately contiguous with both of the sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally-occurring genome of the organism from which it is derived. For example, an isolated nucleic acid can be, without limitation, a recombinant DNA molecule of any length, provided one of the nucleic acid sequences normally found immediately flanking that recombinant DNA molecule in a naturally-occurring genome is removed or absent. Thus, an isolated nucleic acid includes, without limitation, a recombinant DNA that exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences as well as recombinant DNA that is incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, adenovirus, or herpes virus), or into the genomic DNA of a prokaryote or eukaryote. In addition, an isolated nucleic acid can include a recombinant DNA molecule that is part of a hybrid or fusion nucleic acid sequence.

The term "isolated" also includes any non-naturally-occurring nucleic acid since non-naturally-occurring nucleic acid sequences are not found in nature and do not have immediately contiguous sequences in a naturally-occurring genome. For example, non-naturally-occurring nucleic acid such as an engineered nucleic acid is considered to be isolated nucleic acid. Engineered nucleic acid can be made using common molecular cloning or chemical nucleic acid synthesis techniques. Isolated non-naturally-occurring nucleic acid can be independent of other sequences, or incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, adenovirus, or herpes virus), or the genomic DNA of a prokaryote or eukaryote. In

addition, a non-naturally-occurring nucleic acid can include a nucleic acid molecule that is part of a hybrid or fusion nucleic acid sequence.

It will be apparent to those of skill in the art that a nucleic acid existing among hundreds to millions of other nucleic acid molecules within, for example, cDNA or genomic libraries, or gel slices containing a genomic DNA restriction digest is not to be considered an isolated nucleic acid.

Any isolated nucleic acid having a nucleic acid sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 is within the scope of the invention. For convenience, these nucleic acid sequences will be referred to collectively as the IEG nucleic acid group. In addition, any isolated nucleic acid having a nucleic acid sequence at least about 60 percent identical (e.g., at least about 65, 70, 75, 80, 85, 90, 95, or 99 percent identical) to a sequence set forth in the IEG nucleic acid group is within the scope of the invention. For the purpose of this invention, the percent identity between a sequence set forth in the IEG nucleic acid group (designated a template sequence) and any other nucleic acid sequence is calculated as follows. First, the two nucleic acid sequences are aligned using the MEGALIGN® (DNASTAR, Madison, WI, 1997) sequence alignment software following the Jotun Heim algorithm with the default settings. Second, the number of matched positions between the two aligned nucleic acid sequences is determined. A matched position refers to a position in which identical bases occur at the same position as aligned by the MEGALIGN® sequence alignment software. Third, the number of matched positions is divided by the total number of bases in the template sequence, and the resulting value multiplied by 100 to obtain the percent identity. If the obtained percent identity is greater than or equal to about 60 percent for a particular nucleic acid sequence, then that particular nucleic acid sequence is a nucleic acid sequence at least about 60 percent identical to a sequence set forth in the IEG nucleic acid group.

Any isolated nucleic acid having a nucleic acid sequence that encodes an amino acid sequence at least about 60 percent identical (e.g., at least about 65, 70, 75, 80, 85, 90, 95, or 99

percent identical) to the sequence set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, or 62 is within the scope of the invention. For convenience, the amino acid sequences set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, and 62 will be referred to collectively as the IEG amino acid group. For the purpose of this invention, the percent identity between a sequence set forth in the IEG amino acid group (designated a template sequence) and any other amino acid sequence is calculated as follows. First, the two amino acid sequences are aligned using the MEGALIGN® (DNASTAR, Madison, WI, 1997) sequence alignment software following the Jotun Heim algorithm with the default settings. Second, the number of matched positions between the two aligned amino acid sequences is determined. A matched position refers to a position in which identical residues occur at the same position as aligned by the MEGALIGN® sequence alignment software. Third, the number of matched positions is divided by the total number of amino acid residues in the template sequence, and the resulting value multiplied by 100 to obtain the percent identity. If the obtained percent identity is greater than or equal to about 60 percent for a particular amino acid sequence, then that particular amino acid sequence is an amino acid sequence at least about 60 percent identical to a sequence set forth in the IEG amino acid group.

Any isolated nucleic acid having a nucleic acid sequence that encodes an amino acid sequence at least five amino acids in length also is within the scope of the invention provided the encoded amino acid sequence has at least three different amino acid residues, and is identical to a contiguous portion of sequence set forth in a sequence within the IEG amino acid group.

Further, any isolated nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base is within the scope of the invention provided the isolated nucleic acid is at least about 12 bases in length (e.g., at least about 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, or 60 bases in length), and hybridizes, under hybridization conditions, to the sense or antisense strand of a nucleic acid having a sequence as set forth in the IEG nucleic acid group. The hybridization conditions can be moderately or highly stringent hybridization conditions.

For the purpose of this invention, moderately stringent hybridization conditions mean the hybridization is performed at about 42°C in a hybridization solution containing 25 mM KPO_4 (pH7.4), 5X SSC, 5X Denharts solution, 50 μg/ml denatured, sonicated salmon sperm DNA, 50% formamide, 10% Dextran sulfate, and 1-15 ng/ml probe (>$5x10^7$ cpm/μg), while the washes are performed at about 50°C with a wash solution containing 2X SSC and 0.1% SDS.

Highly stringent hybridization conditions mean the hybridization is performed at about 42°C in a hybridization solution containing 25 mM KPO_4 (pH7.4), 5X SSC, 5X Denharts solution, 50 μg/ml denatured, sonicated salmon sperm DNA, 50% formamide, 10% Dextran sulfate, and 1-15 ng/ml probe (>$5x10^7$ cpm/μg), while the washes are performed at about 65°C with a wash solution containing 0.2X SSC and 0.1% SDS.

Nucleic acid within the scope of the invention can be identified and obtained using any method including, without limitation, common molecular cloning and chemical nucleic acid synthesis techniques. For example, PCR can be used to obtain a nucleic acid having a nucleic acid sequence at least about 60 percent identical (e.g., at least about 65, 70, 75, 80, 85, 90, 95, or 99 percent identical) to a sequence set forth in the IEG nucleic acid group. PCR refers to a procedure or technique in which target nucleic acid is amplified in a manner similar to that described in U.S. Patent No. 4,683,195, and subsequent modifications of the procedure described therein. Generally, sequence information from the ends of the region of interest or beyond are used to design oligonucleotide primers that are identical or similar in sequence to opposite strands of a potential template to be amplified. Using PCR, a nucleic acid sequence can be amplified from RNA or DNA. For example, a nucleic acid sequence can be isolated by PCR amplification from total cellular RNA, total genomic DNA, and cDNA as well as from bacteriophage sequences, plasmid sequences, viral sequences, and the like. When using RNA as a source of template, reverse transcriptase can be used to synthesize complimentary DNA strands.

Nucleic acid within the scope of the invention also can be obtained by mutagenesis. For example, a nucleic acid sequence set forth in the IEG nucleic acid group can be mutated using common molecular cloning techniques (e.g., site-directed mutageneses). Possible mutations

include, without limitation, deletions, insertions, and base substitutions, as well as combinations of deletions, insertions, and base substitutions.

In addition, nucleic acid and amino acid databases (e.g., GenBank®) can be used to identify and obtain a nucleic acid within the scope of the invention. For example, any nucleic acid sequence having some homology to a sequence set forth in the IEG nucleic acid group, or any amino acid sequence having some homology to a sequence set forth in the IEG amino acid group can be used as a query to search GenBank®.

Further, nucleic acid hybridization techniques can be used to identify and obtain a nucleic acid within the scope of the invention. Briefly, any nucleic acid having some homology to a sequence set forth in the IEG nucleic acid group, or fragment thereof, can be used as a probe to identify a similar nucleic acid by hybridization under conditions of moderate to high stringency. Such similar nucleic acid then can be isolated, sequenced, and analyzed to determine whether they are within the scope of the invention as described herein.

Hybridization can be done by Southern or Northern analysis to identify a DNA or RNA sequence, respectively, that hybridizes to a probe. The probe can be labeled with a radioisotope such as ^{32}P, an enzyme, digoxygenin, or by biotinylation. The DNA or RNA to be analyzed can be electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with the probe using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook *et al.*, (1989) Molecular Cloning, second edition, Cold Spring harbor Laboratory, Plainview, NY. Typically, a probe is at least about 20 nucleotides in length. For example, a probe corresponding to a 20 nucleotide sequence set forth in the IEG amino acid group. can be used to identify a nucleic acid identical to or similar to a nucleic acid sequence set forth in the IEG nucleic acid group. In addition, probes longer or shorter than 20 nucleotides can be used.

Any cell containing an isolated nucleic acid within the scope of the invention is itself within the scope of the invention. This includes, without limitation, prokaryotic and eukaryotic cells. It is noted that cells containing an isolated nucleic acid of the invention are not required to express the isolated nucleic acid. In addition, the isolated nucleic acid can be integrated into the

genome of the cell or maintained in an episomal state. In other words, cells can be stably or transiently transfected with an isolated nucleic acid of the invention.

Any method can be used to introduce an isolated nucleic acid into a cell. In fact, many methods for introducing nucleic acid into a cell, whether *in vivo* or *in vitro*, are well known to those skilled in the art. For example, calcium phosphate precipitation, electroporation, heat shock, lipofection, microinjection, and viral-mediated nucleic acid transfer are common methods that can be used to introduce nucleic acid into a cell. In addition, naked DNA can be delivered directly to cells *in vivo* as describe elsewhere (U.S. Patent Number 5,580,859 and U.S. Patent Number 5,589,466 including continuations thereof). Further, nucleic acid can be introduced into cells by generating transgenic animals.

Transgenic animals can be aquatic animals (such as fish, sharks, dolphin, and the like), farm animals (such as pigs, goats, sheep, cows, horses, rabbits, and the like), rodents (such as rats, guinea pigs, and mice), non-human primates (such as baboon, monkeys, and chimpanzees), and domestic animals (such as dogs and cats). Several techniques known in the art can be used to introduce nucleic acid into animals to produce the founder lines of transgenic animals. Such techniques include, without limitation, pronuclear microinjection (U.S. Patent No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten *et al.*, *Proc. Natl. Acad. Sci., USA,* 82:6148-6152 (1985)); gene transfection into embryonic stem cells (Gossler A *et al.*, *Proc Natl Acad Sci USA* 83:9065-9069 (1986)); gene targeting into embryonic stem cells (Thompson *et al.*, *Cell*, 56:313-321 (1989)); nuclear transfer of somatic nuclei (Schnieke AE *et al.*, *Science* 278:2130-2133 (1997)); and electroporation of embryos.

For a review of techniques that can be used to generate and assess transgenic animals, skilled artisans can consult Gordon (*Intl. Rev. Cytol.,* 115:171-229 (1989)), and may obtain additional guidance from, for example: Hogan *et al.*, "Manipulating the Mouse Embryo" Cold Spring Harbor Press, Cold Spring Harbor, NY (1986); Krimpenfort *et al.*, *Bio/Technology,* 9:844-847 (1991); Palmiter *et al.*, *Cell, 41*:343-345 (1985); Kraemer *et al.*, "Genetic Manipulation of the Early Mammalian Embryo" Cold Spring Harbor Press, Cold Spring Harbor, NY (1985); Hammer *et al.*, *Nature,* 315:680-683 (1985); Purscel *et al.*, *Science,* 244:1281-1288

(1986); Wagner *et al.*, U.S. Patent No. 5,175,385; and Krimpenfort *et al.*, U.S. Patent No. 5,175,384.

Any method can be used to identify cells that contain an isolated nucleic acid within the scope of the invention. For example, PCR and nucleic acid hybridization techniques such as Northern and Southern analysis can be used. In some cases, immunohistochemistry and biochemical techniques can be used to determine if a cell contains a particular nucleic acid by detecting the expression of a polypeptide encoded by that particular nucleic acid. For example, detection of polypeptide X-immunoreactivity after introduction of an isolated nucleic acid containing a cDNA that encodes polypeptide X into a cell that does not normally express polypeptide X can indicate that that cell not only contains the introduced nucleic acid but also expresses the encoded polypeptide X from that introduced nucleic acid. In this case, the detection of any enzymatic activities of polypeptide X also can indicate that that cell contains the introduced nucleic acid and expresses the encoded polypeptide X from that introduced nucleic acid.

In addition, any method can be used to force a cell to express an encoded amino acid sequence from a nucleic acid. Such methods are well known to those skilled in the art, and include, without limitation, constructing a nucleic acid such that a regulatory element drives the expression of a nucleic acid sequence that encodes a polypeptide. Typically, regulatory elements are DNA sequences that regulate the expression of other DNA sequences at the level of transcription. Such regulatory elements include, without limitation, promoters, enhancers, and the like. Further, any methods can be used to identifying cells that express an amino acid sequence from a nucleic acid. Such methods are well known to those skilled in the art, and include, without limitation, immunocytochemistry, Northern analysis, and RT-PCR.

The term "substantially pure" as used herein with reference to a polypeptide means the polypeptide is substantially free of other polypeptides, lipids, carbohydrates, and nucleic acid with which it is naturally associated. Thus, a substantially pure polypeptide is any polypeptide that is removed from its natural environment and is at least 60 percent free, preferably 75 percent free, and most preferably 90 percent free from other components with which it is naturally

associated. Typically, a substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel.

Any substantially pure polypeptide having an amino acid sequence encoded by a nucleic acid within the scope of the invention is itself within the scope of the invention. In addition, any substantially pure polypeptide having an amino acid sequence at least about 60 percent (e.g., at least about 65, 70, 75, 80, 85, 90, 95, or 99 percent) identical to a sequence set forth in the IEG amino acid group is within the scope of the invention. The percent identity between particular amino acid sequences is determined as described herein.

Any method can be used to obtain a substantially pure polypeptide. For example, one skilled in the art can use common polypeptide purification techniques such as affinity chromotography and HPLC as well as polypeptide synthesis techniques. In addition, any material can be used as a source to obtain a substantially pure polypeptide. For example, tissue from wild-type or transgenic animals can be used as a source material. In addition, tissue culture cells engineered to overexpress a particular polypeptide of interest can be used to obtain substantially pure polypeptide. Further, a polypeptide within the scope of the invention can be "engineered" to contain an amino acid sequence that allows the polypeptide to be captured onto an affinity matrix. For example, a tag such as c-myc, hemagglutinin, polyhistidine, or Flag® tag (Kodak) can be used to aid polypeptide purification. Such tags can be inserted anywhere within the polypeptide including at either the carboxyl or amino termini. Other fusions that could be useful include enzymes that aid in the detection of the polypeptide, such as alkaline phosphatase.

The term "antibody" as used herein refers to intact antibodies as well as antibody fragments that retain some ability to selectively bind an epitope. Such fragments include, without limitation, Fab, F(ab')2, and Fv antibody fragments. The term "epitope" refers to an antigenic determinant on an antigen to which the paratope of an antibody binds. Epitopic determinants usually consist of chemically active surface groupings of molecules (e.g., amino acid or sugar residues) and usually have specific three dimensional structural characteristics as well as specific charge characteristics.

Any antibody having specific binding affinity for an amino acid sequence encoded by a nucleic acid within the scope of the invention is itself within the scope of the invention. Thus, any monoclonal or polyclonal antibody having specific binding affinity for an amino acid sequence set forth in the IEG amino acid group is within the scope of the invention. Such antibodies can be used in immunoassays in liquid phase or bound to a solid phase. For example, the antibodies of the invention can be used in competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays include the radioimmunoassay (RIA) and the sandwich (immunometric) assay.

Antibodies within the scope of the invention can be prepared using any method. For example, any substantially pure polypeptide provided herein, or fragment thereof, can be used as an immunogen to elicit an immune response in an animal such that specific antibodies are produced. Thus, an intact full-length polypeptide or fragments containing small peptides can be used as an immunizing antigen. In addition, the immunogen used to immunize an animal can be chemically synthesized or derived from translated cDNA. Further, the immunogen can be conjugated to a carrier polypeptide, if desired. Commonly used carriers that are chemically coupled to an immunizing polypeptide include, without limitation, keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid.

The preparation of polyclonal antibodies is well-known to those skilled in the art. See, e.g., Green *et al.*, *Production of Polyclonal Antisera*, in IMMUNOCHEMICAL PROTOCOLS (Manson, ed.), pages 1-5 (Humana Press 1992) and Coligan *et al.*, *Production of Polyclonal Antisera in Rabbits, Rats, Mice and Hamsters*, in CURRENT PROTOCOLS IN IMMUNOLOGY, section 2.4.1 (1992). In addition, those of skill in the art will know of various techniques common in the immunology arts for purification and concentration of polyclonal antibodies, as well as monoclonal antibodies (Coligan, *et al.*, Unit 9, Current Protocols in Immunology, Wiley Interscience, 1994).

The preparation of monoclonal antibodies also is well-known to those skilled in the art. See, e.g., Kohler & Milstein, *Nature* 256:495 (1975); Coligan *et al.*, sections 2.5.1-2.6.7; and Harlow *et al.*, ANTIBODIES: A LABORATORY MANUAL, page 726 (Cold Spring

Harbor Pub. 1988). Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen, verifying the presence of antibody production by analyzing a serum sample, removing the spleen to obtain B lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures. Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, e.g., Coligan *et al.* , sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes *et al.*, *Purification of Immunoglobulin G (IgG)* , in METHODS IN MOLECULAR BIOLOGY, VOL. 10, pages 79-104 (Humana Press 1992).

In addition, methods of *in vitro* and *in vivo* multiplication of monoclonal antibodies is well-known to those skilled in the art. Multiplication *in vitro* can be carried out in suitable culture media such as Dulbecco's Modified Eagle Medium or RPMI 1640 medium, optionally replenished by mammalian serum such as fetal calf serum, or trace elements and growth-sustaining supplements such as normal mouse peritoneal exudate cells, spleen cells, and bone marrow macrophages. Production *in vitro* provides relatively pure antibody preparations and allows scale-up to yield large amounts of the desired antibodies. Large scale hybridoma cultivation can be carried out by homogenous suspension culture in an airlift reactor, in a continuous stirrer reactor, or in immobilized or entrapped cell culture. Multiplication *in vivo* may be carried out by injecting cell clones into mammals histocompatible with the parent cells (e.g., osyngeneic mice) to cause growth of antibody-producing tumors. Optionally, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. After one to three weeks, the desired monoclonal antibody is recovered from the body fluid of the animal.

The antibodies within the scope of the invention also can be made using non-human primates. General techniques for raising therapeutically useful antibodies in baboons can be

found, for example, in Goldenberg *et al.*, International Patent Publication WO 91/11465 (1991) and Losman *et al.*, *Int. J. Cancer* 46:310 (1990).

Alternatively, the antibodies can be "humanized" monoclonal antibodies. Humanized monoclonal antibodies are produced by transferring mouse complementarity determining regions (CDRs) from heavy and light variable chains of the mouse immunoglobulin into a human variable domain, and then substituting human residues in the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions when treating humans. General techniques for cloning murine immunoglobulin variable domains are described, for example, by Orlandi *et al.*, *Proc. Nat'l. Acad. Sci. USA* 86:3833 (1989). Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al.*, *Nature* 321:522 (1986); Riechmann *et al.*, *Nature* 332:323 (1988); Verhoeyen *et al.*, *Science* 239:1534 (1988); Carter *et al.*, *Proc. Nat'l. Acad. Sci. USA* 89:4285 (1992); Sandhu, *Crit. Rev. Biotech.* 12:437 (1992); and Singer *et al.*, *J. Immunol.* 150:2844 (1993).

Antibodies of the present invention also may be derived from human antibody fragments isolated from a combinatorial immunoglobulin library. See, for example, Barbas *et al.*, METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, VOL. 2, page 119 (1991) and Winter *et al.*, *Ann. Rev. Immunol.* 12: 433 (1994). Cloning and expression vectors that are useful for producing a human immunoglobulin phage library can be obtained, for example, from STRATAGENE Cloning Systems (La Jolla, CA).

In addition, antibodies of the present invention may be derived from a human monoclonal antibody. Such antibodies are obtained from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain loci are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens and can be used to produce human antibody-secreting

hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green *et al.*, *Nature Genet.* 7:13 (1994); Lonberg *et al.*, *Nature* 368:856 (1994); and Taylor *et al.*, *Int. Immunol.* 6:579 (1994).

Antibody fragments of the present invention can be prepared by proteolytic hydrolysis of an intact antibody or by the expression of a nucleic acid encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of intact antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg (U.S. Patent Nos. 4,036,945 and 4,331,647). See also Nisonhoff *et al.*, *Arch. Biochem. Biophys.* 89:230 (1960); Porter, *Biochem. J.* 73:119 (1959); Edelman *et al.*, METHODS IN ENZYMOLOGY, VOL. 1, page 422 (Academic Press 1967); and Coligan *et al.* at sections 2.8.1-2.8.10 and 2.10.1-2.10.4.

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used provided the fragments retain some ability to selectively bind its epitope.

For example, Fv fragments comprise an association of V_H and V_L chains. This association may be noncovalent, as described in Inbar *et al.*, *Proc. Nat'l. Acad. Sci. USA* 69:2659 (1972). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. See, e.g., Sandhu, *supra.* Preferably, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding polypeptides (sFv) are prepared by constructing a nucleic acid construct encoding the V_H and V_L domains connected by an oligonucleotide. This nucleic acid construct is inserted into an expression vector, which is subsequently introduced

into a host cell such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by Whitlow *et al.*, METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, VOL. 2, page 97 (1991); Bird *et al.*, *Science* 242:423-426 (1988); Ladner *et al.*, U.S. patent No. 4,946,778; Pack *et al.*, *Bio/Technology* 11:1271-77 (1993); and Sandhu, *supra*.

Another form of an antibody fragment is a peptide coding for a single CDR. CDR peptides ("minimal recognition units") can be obtained by constructing nucleic acid constructs that encode the CDR of an antibody of interest. Such constructs are prepared, for example, by using PCR to synthesize the variable region from RNA of antibody-producing cells. See, e.g., Larrick *et al.*, METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, VOL. 2, page 106 (1991).

It is also possible to use anti-idiotype technology to produce monoclonal antibodies that mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region that is the "image" of the epitope bound by the first monoclonal antibody. Such anti-idiotypic monoclonal antibodies can be used to inhibit the activity of the polypeptide containing the original epitope.

The invention also provides cDNA libraries enriched for IEGs. As described herein, such cDNA libraries contain an increased frequency of cDNAs derived from IEGs. Specifically, about 15 percent (e.g., about 20 or 25 percent) of the cDNA clones within the cDNA libraries provided herein are derived from IEGs.

A cDNA library within the scope of the invention can be prepared from any tissue containing cells that express an IEG (e.g., hippocampus tissue). Again, an IEG is a gene whose expression is rapidly increased immediately following a stimulus. The stimulus can be electrical or chemical in nature. For example, cells can be treated with electric shock or chemicals such as kainate. Briefly, cDNA libraries are prepared from the hippocampus of control animals (e.g., rats) as well as from animals that receive a stimulus (e.g., multiple MECS) using, for example, a

phage vector lambda ZAP II (Stratagene). A subtracted library is then prepared using *in vitro* mRNA prepared from a control library and subsequent solution phase hybridization with cDNA prepared from a stimulated library. The control *in vitro* mRNA can be tagged with biotin to permit its removal from solution using avidin beads (Lanahan *et al.*, *Mol. Cell. Biol.* 12:3919-3929 (1992)). cDNA that remains after removal of mRNA/cDNA hybrids can be recloned into, for example, a lambda ZAPII phage vector. Several rounds of subtraction (e.g., two, three, four, or five rounds) can be used to increase the frequency of IEGs. The subtracted library then can be plated and duplicate phage lifts screened with a radiolabeled cDNA probe. Any probe can be used provided it contains at least one nucleic acid sequence derived from an IEG. For example, a probe can be prepared from mRNA obtained from the hippocampus of a stimulated animal. In addition, the mRNA used to make a probe can be subjected to subtractive hybridization such that IEG sequences are enriched. In general, conventional cDNA libraries contain IEGs at a frequency of <1:30,000 cDNAs. For the cDNA libraries enriched for IEGs, however, about 1 in 5 genes can be induced by a stimulus such as MECS. This represents an about 1000 to 10,000 fold enrichment in brain IEGs.

An animal (e.g., human) having a deficiency in a neuron's IEG responsiveness to a stimulus (e.g., a stimulus that influences learning or memory) can be treated using the methods and materials described herein. A stimulus that influences learning or memory can be a multiple MECS treatment. A deficiency in a neuron's IEG responsiveness to a stimulus means the level of IEG responsiveness is not normal. Such deficiencies can be identified by stimulating a sample of cells and measuring the levels of IEG expression. If the levels are not similar to the levels normally observed in a similar tissue sample, then there is a deficiency. It is noted that increased IEG expression as well as decreased IEG expression can be classified as a deficiency provided the levels are not normal.

A deficiency in a neuron's IEG responsiveness to a stimulus can be treated by administering a nucleic acid of the invention to the animal such that the effect of the deficiency is minimized. The administration can be an *in vivo*, *in vitro*, or *ex vivo* administration as described herein. For example, an *in vivo* administration can involve administering a viral vector

to the hippocampal region of an animal, while an *ex vivo* administration can involve extracting cells from an animal, transfecting the cells with the nucleic acid in tissue culture, and then introducing the transfected cells back into the same animal.

In addition, a deficiency in a neuron's IEG responsiveness to a stimulus can be treated by administering a therapeutically effective amount cells containing isolated IEG nucleic acid, substantially pure IEG polypeptides, anti-IEG polypeptide antibodies, or combinations thereof. A therapeutically effective amount is any amount that minimizes the effect of the deficiency while not causing significant toxicity to the animal. Such an amount can be determined by assessing the clinical symptoms associated with the deficiency before and after administering a fixed amount of cells, polypeptides, or antibodies. In addition, the effective amount administered to an animal can be adjusted according to the animal's response and desired outcomes. Significant toxicity can vary for each particular patient and depends on multiple factors including, without limitation, the patient's physical and mental state, age, and tolerance to pain. The cells, polypeptides, or antibodies can be administered to any part of the animal's body including, without limitation, brain, spinal cord, blood stream, muscle tissue, skin, peritoneal cavity, and the like. Thus, these therapeutic agents can be administered by injection (e.g., intravenous, intraperitoneal, intramuscular, subcutaneous, intracavity, or transdermal injection) or by gradual perfusion over time.

Preparations for administration can include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Other vehicles for adminstration include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's intravenous vehicles containing fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Further, a deficiency in a neuron's IEG responsiveness to a stimulus can be treated by administering a therapeutically effective amount of a compound that directly interferes with the translation of IEG nucleic acid. For example, antisense nucleic acid or ribozymes could be used to bind to IEG mRNA or to cleave it. Antisense RNA or DNA molecules bind specifically with a targeted RNA message, interrupting the expression of the mRNA product. The antisense binds to the messenger RNA forming a double stranded molecule that cannot be translated by the cell. Typically, an antisense oligonucleotides is about 15-25 nucleotides in length. In addition, chemically reactive groups, such as iron-linked ethylenediaminetetraacetic acid (EDTA-Fe), can be attached to an antisense oligonucleotide, causing cleavage of the mRNA at the site of hybridization. These and other uses of antisense methods to inhibit the translation of nucleic acid are well known in the art (Marcus-Sakura, *Anal. Biochem.*, 172:289 (1988)).

An oligonucleotide also can be used to stall transcription winding around double-helical DNA and forming a three-strand helix (Maher, *et al.*, *Antisense Res. and Dev.*, 1:227 (1991) and Helene, *Anticancer Drug Design*, 6:569 (1991)).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. By modifying nucleic acid sequences that encode ribozymes, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, *J. Amer. Med. Assn.*, 260:3030 (1988)). There are two basic types of ribozymes namely, tetrahymena-type (Hasselhoff, *Nature*, 334:585 (1988)) and "hammerhead"-type. Tetrahymena-type ribozymes recognize sequences that are four bases in length, while "hammerhead"-type ribozymes recognize sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, "hammerhead"-type ribozymes are preferable to tetrahymena-type ribozymes for inactivating a specific mRNA species. In addition, 18-based recognition sequences are preferable to shorter recognition sequences. These and other uses of antisense methods to inhibit the *in vivo* translation of nucleic acid are well known in the art (DeMesmaeker *et al.*, *Curr. Opin. Struct.*

Biol. 5:343-355 (1995); Gewirtz *et al.*, *Proc. Nat'l. Acad. Sci. U.S.A.*, 93:3161-3163 (1996); and Stein, *Chem. Biol.* 3:319-323 (1996)).

Delivery of nucleic acid, antisense, triplex agents, and ribozymes can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Various viral vectors that can be utilized for gene therapy include adenoviruses, herpesviruses, vaccinia viruses, and retroviruses. A retroviral vector can be a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. In addition, a nucleic acid sequence of interest along with another nucleic acid sequence that encodes a ligand for a receptor on a specific target cell can be inserted into a viral vector to produce a vector that is target specific. For example, retroviral vectors can be made target specific by inserting a nucleic acid sequence that encodes an antibody that binds a specific target antigen. Those of skill in the art can readily ascertain without undue experimentation specific nucleic acid sequences that can be inserted into a retroviral genome to allow target specific delivery of the retroviral vector containing the nucleic acid of the invention.

A colloidal dispersion system can be used to target the delivery of the nucleic acid of the invention. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. Liposomes are artificial membrane vesicles that are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV) that range in size from 0.2-4.0 μm can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. Thus, nucleic acid, intact virions, polypeptides, and antibodies can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley *et al.*, *Trends Biochem. Sci.*, 6:77 (1981)). In addition to mammalian cells, liposomes have been used to deliver nucleic acid to plants, yeast, and bacteria. In order for a liposome to

be an efficient nucleic acid transfer vehicle, the following characteristics should be present: (1) encapsulation of the nucleic acid of interest at high efficiency while not compromising its biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of the nucleic acid (Mannino *et al.*, *Biotechniques*, 6:682 (1988).

The composition of a liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids also can be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine, and distearoylphosphatidylcholine.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand. In general, the compounds bound to the surface of the targeted delivery system will be ligands and receptors that allow the targeted delivery system to find and "home in" on the desired cells. A ligand may be any compound of interest that will bind to another compound, such as a receptor or antibody.

Compounds that modulate IEG expression can be identified by contacting a test compound with an IEG nucleic acid, and determining whether the test compound effects expression. Likewise, compounds that modulate IEG polypeptide activity can be identified by contacting a test compound with an IEG polypeptide, and determining whether the test

compound effects polypeptide activity. Contacting includes in solution and in solid phase, or in a cell. Any type of compound can be used as a test compound including, without limitation, peptides, peptidomimetics, polypeptides, chemical compounds, and biologic agents. In addition, the test compound can be a combinatorial library for screening a plurality of compounds. Compounds identified using the method of the invention can be further evaluated, detected, cloned, sequenced, and the like, either in solution or after binding to a solid support, by any method usually applied to the detection of a specific DNA sequence such as PCR, oligomer restriction (Saiki, *et al.*, *Bio/Technology*, 3:1008-1012, 1985), allele-specific oligonucleotide (ASO) probe analysis (Conner, *et al.*, *Proc. Nat'l. Acad. Sci. USA*, 80:278 (1983), oligonucleotide ligation assays (OLAs; Landegren, *et al.*, *Science*, 241:1077 (1988), and the like.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1 - Construction of subtracted cDNA libraries

The mRNA used to prepare the cDNA libraries was obtained from the hippocampus of adult rats (male or female). Briefly, the hippocampus was dissected from naive or stimulated rats, and rapidly frozen in liquid nitrogen. The stimulation protocol used to stimulate the rats was as follows. Rats were injected intraperitoneally with 50 mg of the protein synthesis inhibitor cycloheximide (50 mg/ml stock in 50% ethanol) per kilogram of body weight 15 minutes prior to initiating repetitions of maximal electroconvulsive seizure (MECS). MECS was induced by passage of a constant current signal by means of an ECT unit (Ugo, Basil). The current signal lasted one second with a frequency of 100 Hz. Each pulse lasted 0.5 milliseconds, and the current was 90 milliamperes. This stimulus caused brief loss of consciousness and a tonic-clonic seizure lasting 30 seconds to one minute. MECS was administered about every 15 minutes for a total of 13 administrations over the course of 2.5 to 3 hours. Thirty (30) minutes after the last MECS, the rats were sacrificed by decapitation.

To collect total RNA, the tissue was homogenized in 4M guanidinium thiocynate using a polytron and then centrifuged through a CsCl cushion. To isolate polyA$^+$ RNA, the resulting total RNA was chromatographed on oligo(dT) columns using a commercial oligo(dT) resin and purification protocol (Fastback, Invitrogen). About 50 naive (control) and 50 stimulated rats were used to generate the polyA$^+$ mRNA needed to make the cDNA libraries and perform the Northern blot analysis.

A nonsubtracted cDNA library was made using polyA$^+$ RNA isolated from rats subjected to MECS. Briefly, cDNA was synthesized and cloned directionally into the Lambda ZAP vector yielding a library containing 3.6 x 10^6 recombinants. This library was designated the 3 hr MECS/CHX library. Differential screening of the 3 hr MECS/CHX library with control and stimulated rat hippocampal cDNA probes yielded several novel IEGs. Analysis of these IEGs revealed that they were relatively abundant.

The 3 hr MECS/CHX library was used as starting material for preparing a subtracted cDNA library highly enriched for IEGs. A subtracted cDNA library highly enriched for IEGs can allow for the detection of lower abundance novel IEGs. To make a subtracted cDNA library, DNA template was prepared from the 3 hr MECS/CHX library as follows.

The 3 hr MECS/CHX library was amplified and plated on 15 cm NZCYM agarose plates at a density of about 50,000 phage/plate. A total of 1.85 x 10^6 phage were plated on a total of 37 plates. The plates were overlaid with Suspension Media (SM) and the phage particles eluted by swirling at 4°C overnight. The lysate was collected, and chloroform added to a final concentration of 5%. The lysate was clarified by centrifugation, and the phage containing supernatant collected and stored at 4°C. A 300 ml aliquot of the lysate was treated with RNaseA (final concentration of 1 μg/μl) and DNase I (final concentration of 1 μg/μl) for three hours at 37°C. Polyethylene glycol (PEG 6000) was added to a concentration of 10%, and NaCl added to a concentration of 1 M. After mixing well, the lysate was stored at 4°C overnight to allow phage particles to precipitate. Phage particles were pelleted by centrifugation, resuspended in 20 ml of SM, and stored at 4°C. Phage particles were lysed by adding EDTA to a concentration of 10 mM and SDS to a concentration of 0.2% followed by a 20 minute incubation at 68°C. Polypeptides

were removed by two extractions with phenol/chloroform/isoamyl alcohol (50:48:2) followed by two extractions with chloroform/isoamyl alcohol (24:1). The phage DNA contained within 40 ml of lysate was precipitated by adding 1/10th volume of 3M NaOAc (pH 5.2) followed by the addition of 2 volumes of 100% ethanol. After mixing, the solution was incubated at -20°C overnight. DNA was pelleted by centrifugation, resuspended in 10 mM Tris, 1 mM EDTA pH 7.5 (TE), and reprecipitated overnight. After this second precipitation, the DNA was pelleted by centrifugation and resuspended in 12 ml of 10 mM Tris (pH 7.5), 5 mM EDTA, 300 mM NaCl. To remove residual RNA, RNase A (final concentration of 50 μg/ml) was added followed by incubation at 37°C for 1 hour. To remove RNase A, SDS (final concentration of 0.5%) and then Proteinase K (final concentration of 50 μg/ml) was added followed incubation at 37°C for 1 hour. The DNA lysate was extracted twice with phenol/chloroform/isoamyl alcohol (50:48:2) followed by one extraction with chloroform/isoamyl alcohol (24:1). After this extraction, the DNA lysate was dialyzed against 12 liters of TE for 2 days at 4°C. The 300 ml aliquot of phage lysate yielded 7254 μg of phage DNA. This phage DNA was then used to prepare *in vitro* polyA$^+$ RNA (cRNA).

To prepare *in vitro* cRNA, the phage DNA template was linearized at the 3' end of the cDNA insert using the restriction enzyme XhoI. Briefly, 1 mg of phage DNA was digested with 1000 U of XhoI for three hours at 37°C. After the three hour incubation, an additional 1000 U of XhoI was added and the 37°C incubation continued an additional three hours. XhoI was removed by adding SDS to 0.5% and Proteinase K to 50 μg/ml followed by incubation at 37°C for one hour. Polypeptides were removed by three extractions with phenol/chloroform/isoamyl alcohol (50:48:2) followed by one extraction with chloroform/isoamyl alcohol (24:1). The DNA was precipitated with 1/10th volume 3M NaOAc (pH 5.2) and 2 volumes 100% ethanol. The DNA was pelleted by centrifugation and resuspended in 500 μl TE (1.58 mg/ml final DNA concentration).

This linearized DNA was used as template to prepare *in vitro* cRNA from the sense strand of the cDNA inserts. This cRNA is representative of the initial *in vivo* population of RNA in the MECS/cycloheximide treated rat hippocampus. Forty (40) μg of DNA template was

incubated with 40 mM Tris (pH 7.5), 6 mM $MgCl_2$, 2 mM spermidine, 10 mM NaCl, 10 mM DTT, 1 U/μl RNasin, 500 μM ATP, 500 μM CTP, 500 μM GTP, 500 μM UTP, and 2 U/μl T3 RNA polymerase in a final volume of 300 μl for two hours at 40°C. After two hours, an additional 2 U/μl of T3 RNA polymerase was added, and the reaction incubated for an additional two hours at 37°C for a total time of four hours. The DNA template was removed by adding DNaseI (2 U/μg of template) and incubating the mixture at 37°C for an hour. Polypeptides were removed by two extractions with phenol/chloroform/isoamyl alcohol (50:48:2) followed by one extraction with chloroform/isoamyl alcohol (24:1). The cRNA was precipitated at 20°C with one half volume 7.5 M NH_4OAc and 2 volumes 100% ethanol. The cRNA was pelleted and resuspended in TE. The cRNA was chromatographed on sephadex G-50 columns (NICK columns; Pharmacia) to remove free nucleotides and the concentration of cRNA determined by UV absorbance at 260 A. Thirty (30) μg of DNA template yielded 68.6 μg of cRNA. The cRNA was either stored frozen at -20°C or precipitated with 1/10th volume 2 M KOAc (pH 5) and 2 volumes 100% ethanol. The 68.6 μg of cRNA was further purified using oligo(dT) column chromotography to select $polyA^+$ cRNA. The cRNA was bound to oligo(dT) under high salt conditions, rinsed with low salt conditions, and eluted with TE (pH 7.5). This eluted cRNA was again passed over an oligo(dT) column under high salt conditions, rinsed with low salt conditions, and the $polyA^+$ cRNA eluted with TE (pH 7.5). The two passes on oligo(dT) cellulose yielded 34.2 μg of $polyA^+$ cRNA. This $polyA^+$ cRNA was then used as template for synthesis of first strand cDNA that was then subtracted against control brain and liver $polyA^+$ RNA.

Two cDNA synthesis reactions were performed to prepare first strand cDNA from the $polyA^+$ cRNA. One involved using 2 μg of cRNA with a small amount of ^{32}P-dCTP to allow for the analysis of subtraction efficiency, and the other involved using 5 μg of cRNA with no radioactive dNTPs. The radioactive cDNA synthesis reaction was as follows. First, 2 μl cRNA (1 μg/μl in TE), 1 μl Xho(dT) primer (1.4 μg/μl), and 8 μl water was combined, and the mixture was incubated at 70°C for ten minutes, quickly spun, and placed on ice. Second, 1 μl RNasin (40 U/μl), 5 μl 5X Reaction Buffer (BRL), 2.5 μl 0.1M DTT, 1.5 μl dNTP mix, and 2 μl ^{32}P

dCTP (3000 Ci/mmole) was added, and the mixture was incubated at room temperature for ten minutes. The dNTP mix contained 10 mM of each dATP, dGTP, and dTTP as well as 5 mM of methyl dCTP. After incubation, 2 µl of Superscript/MMLV RT mix (1:1) was added, and the mixture (25 µl total volume) was incubated at room temperature for five minutes followed by a 90 minute incubation at 40°C. The nonradioactive cDNA synthesis reaction was as follows. First, 5 µl cRNA (1 µg/µl in TE), 2 µl Xho(dT) primer (1.4 µg/µl), and 3 µl water was combined, and the mixture was incubated at 70°C for ten minutes, quickly spun, and placed on ice. Second, 1 µl RNasin (40 U/µl), 5 µl 5X Reaction Buffer (BRL), 2.5 µl 0.1M DTT, and 1.5 µl dNTP mix was added, and the mixture was incubated at room temperature for ten minutes. After incubation, 5 µl of Superscript/MMLV RT mix (1:1) was added, and the mixture (25 µl total volume) was incubated at room temperature for five minutes followed by a 90 minute incubation at 40°C.

After completion, 3.2 µl of 0.5 M EDTA (pH 8.0) was added to the radioactive reaction, and then the radioactive and nonradioactive reactions were combined. For subtractive hybridizations, it was necessary to remove the cRNA template by alkaline hydrolysis. This was done by adding 25 µl of TE (pH 7.5) and 5.8 µl of 2 M NaOH. This resulted in a 20 mM final concentration of EDTA and a 138 mM final concentration of NaOH. The mixture was heated for 30 minutes at 68 to 70°C, and then 12.2 µl of 1 M Tris (pH 7.5) and 5.8 µl of 2 N HCl was added to neutralize the reaction. The final volume was 100 µl of which 2 µl was removed and counted to determine the percent incorporation of ^{32}P-dCTP into cDNA. This analysis revealed that 7000 ng of cRNA was converted to 2598 ng of first strand cDNA. This first strand cDNA was subtracted against adult rat brain and liver polyA$^+$ RNA.

For the subtractive hybridizations, the first strand cDNA was chromatographed on a sephadex G-50 column (NICK, Pharmacia) to remove unincorporated dNTPs, especially the unincorporated ^{32}P-dCTP in order to allow the efficiency of subtraction to be followed. After the cDNA was eluted from the NICK column, it was mixed with 60 µg of adult rat brain polyA$^+$ RNA that was coupled to biotin (2X Bio RNA). The cDNA and polyA$^+$ RNA mixture was precipitated by adding 1/10th volume 3M NaOAc (pH 5.2) and 2 volumes 100% ethanol. This

mixture then was pelleted and resuspended in 20 µl TE (pH 7.5) and 20 µl 2X Subtraction Hybridization Buffer (100 mM Hepes (pH 7.6), 0.4% SDS, 4 mM EDTA, 1 M NaCl). The resuspended cDNA and polyA$^+$ RNA mixture was then incubated at 95°C for two minutes, quickly spun, and submerged in a 60°C water bath for 48 hours to allow hybrids to form between the cDNA and biotinylated polyA$^+$ RNA (BioRNA).

The cDNA/BioRNA complexes were removed as follows. First, 40 µl 1X Subtraction Hybridization Buffer lacking SDS and 20 µl Strepavidin (1 mg/ml) was added, and the resulting mixture incubated at room temperature for ten minutes. After incubation, the cDNA/BioRNA complexes were removed by extraction with phenol/chloroform/isoamyl alcohol. The phenol phase was back-extracted with 1X Subtraction Hybridization Buffer lacking SDS, and the aqueous phases pooled. Once pooled, 20 µl Strepavidin (1 mg/ml) was added, and the resulting mixture incubated at room temperature for ten minutes. After incubation, remaining cDNA/BioRNA complexes were removed by extraction with phenol/chloroform/isoamyl alcohol. The phenol phase was back-extracted with 1X Subtraction Hybridization Buffer lacking SDS, and the aqueous phases pooled. The pooled aqueous phases (about 400 µl) were extracted with chloroform/isoamyl alcohol. At this point, an aliquot of the aqueous phase was counted to determine the amount of cDNA remaining. Results revealed that 78% of the starting cDNA was removed with 22% remaining (572 ng).

To perform a second round of subtraction, the aqueous phase (about 400 µl) containing the non-hybridizing first strand cDNA was mixed with 30 µg of adult rat brain polyA$^+$ RNA coupled to biotin and 30 µg of adult rat liver polyA$^+$ RNA coupled to biotin. The cDNA and biotinylated polyA$^+$ RNA was co-precipitated and hybridized as described for the first round. In addition, the cDNA/BioRNA complexes were removed as described above, and the percentage of non-hybridizing cDNA remaining was determined. Results revealed that two rounds of subtraction removed 87.5% of the starting cDNA with 12.5% of the starting cDNA remaining.

A third round of subtraction similar to the second round was performed using the remaining cDNA. Analysis of the remaining cDNA revealed that the three rounds of subtraction had removed 90% of the starting cDNA leaving 10% of the starting cDNA (255 ng).

The remaining single stranded cDNA was used to synthesize double stranded cDNA for the subtracted cDNA library. First, the single stranded cDNA (300 µl) was alkali treated to remove any remaining RNA as follows. The final concentration of EDTA was adjusted to 20 mM by addition of 13 µl of 0.5M EDTA, and then 20 µl of 2M NaOH (120 mM final concentration) was added. This mixture was incubated at 68°C for 30 minutes and then neutralized by adding 40 µl 1 M Tris (pH 7.5) and 20 µl 2 N HCl. The cDNA was precipitated by adding 10 µl glycogen (10 mg/ml), 1/10th volume 3M NaOAc (pH 5.2), and 2 volumes ethanol. The cDNA then was pelleted, resuspended in 100 µl of TE (pH 7.5), and purified on a sephadex G-50 column (NICK, Pharmacia). The purified cDNA was re-precipitated using glycogen, pelleted, and resuspended in TE (pH 7.5) as described. Second, 50 µl resuspended cDNA (single stranded, subtracted cDNA), 20 µl 5X Sequenase Buffer, and 13 µl water was combined, and the mixture incubated at 65°C for five minutes, 37°C for ten minutes, and room temperature for 30 minutes. After incubation, 5 µl dNTP mix, 5 µl 0.1 M DTT, 2 µl Sequenase (13 U/µl), and 2 µl Klenow (5 U/µl) was added, and the mixture (100 µl final volume) incubated at 37°C for one hour. The dNTP mix contained 10 mM dATP, 10 mM dCTP, 10 mM dGTP, and 10 mM dTTP. The reaction was terminated by adding 3 µl of 0.5 M EDTA (pH 8.0) followed by two extractions with phenol/chloroform/isoamyl alcohol and a final extraction with chloroform/isoamyl alcohol. The double stranded cDNA was ethanol precipitated, pelleted by centrifugation, and resuspended in 86 µl TE (pH 7.5).

The double stranded cDNA was then restriction digested as follows. Eighty-six (86) µl cDNA, 10 µl 10X EcoRI Reaction Buffer (NEB), 2 µl EcoRI (20 U/µl), and 2 µl XhoI (20 U/µl) was combined, and the mixture (100 µl final volume) incubated at 37°C for one hour. After this incubation, an additional 2 µl EcoRI (20 U/µl) and 2 µl XhoI (20 U/µl) was added, and the mixture again incubated at 37°C for one hour. After digestion, the reaction was extracted twice with phenol/chloroform/isoamyl alcohol followed by one chloroform/isoamyl alcohol extraction. The digested cDNA was precipitated with ethanol, pelleted by centrifugation, and resuspended in 40 µl of 10 mM Tris (pH 7.5), 1 mM EDTA, 100 mM NaCl, and 20 µl loading buffer. The cDNA was divided into two aliquots, and each aliquot was size-fractionated on a 1 ml BioGel A-

50m column. The columns were rinsed with 10 mM Tris (pH 7.5), 1 mM EDTA, and 100 mM NaCl, with 50 µl fractions being collected. One column was run to select for only relatively long cDNAs while the other was run to select for all cDNAs. These separate pools were then extracted twice with phenol/chloroform/isoamyl alcohol followed by one chloroform/isoamyl alcohol extraction. The cDNA was precipitated by adding 5 µl yeast tRNA (1 µg/µl) and 2 volumes of 100% ethanol. The cDNA was pelleted by centrifugation and directionally cloned into lambda phage UniZAP as follows. For the regular cDNAs (all sizes), 4 µl water, 2 µl 5X Ligase Buffer (BRL), 2 µl UniZAP (500 ng/µl), and 2 µl T4 DNA Ligase (10 U/µl) was added to the pelleted cDNA, and the mixture (10 µl final volume) incubated at 14°C overnight. For the large cDNAs, 2 µl water, 1 µl 5X Ligase Buffer (BRL), 1 µl UniZAP (500 ng/µl), and 1 µl T4 DNA Ligase (10 U/µl) was added to the pelleted cDNA, and the mixture (5 µl final volume) incubated at 14°C overnight. The ligated cDNA was then packaged using packing extracts (Stratagene) and titered on XL1-Blue MRF cells. The subtracted 3 hr MECS/CHX cDNA library containing large cDNAs (designated IEG-Lg cDNA library) had 239,000 recombinants, and the subtracted 3 hr MECS/CHX cDNA library containing regular cDNAs (designated IEG-Reg cDNA library) had 4,992,000 recombinants. A portion of each library was rescued as pBluescript plasmid, and the cDNA inserts analyzed. Of 46 plasmids analyzed from the IEG-Lg cDNA library, all contained cDNA inserts with the average insert size being 1.36 kilobases. Of 44 plasmids analyzed from the IEG-Reg cDNA library, 43 contained cDNA inserts with the average insert size being 0.9 kilobases.

Duplicate southern blots containing cDNA from the 44 plasmids analyzed from the IEG-Reg cDNA library were probed with control and stimulated subtracted ^{32}P-oligolabeled cDNA from rat hippocampus. Eleven of the 44 cDNA inserts gave a clear differential signal that was stronger with the 3 hour MECS/CHX cDNA probe than with the control cDNA probe. This result indicates that 1 in 4 of the clones in the IEG-Reg cDNA library is derived from an IEG.

Example 2 - Preparation of subtracted cDNA probes

Subtracted cDNA was prepared using exactly the same protocol described in example 1 with the exception that rather than *in vitro* cRNA being used as the template for cDNA synthesis, polyA$^+$ RNA derived from control rat hippocampi or rat hippocampi from rats treated with the 3 hour MECS protocol was used. After first strand cDNA synthesis, the RNA template was denatured by alkaline hydrolysis, and the free nucleotides removed by chromotography on sephadex G-50 (NICK, Pharmacia). The cDNA was precipitated using 1/10th volume 3M NaOAc (pH 5.2), 2 µl glycogen (20 mg/ml), and 2 volumes ethanol, pelleted by centrifugation, and resuspended in TE (pH 7.5). The final concentration was 25 ng/µl. The single strand of cDNA was labeled to high specific activity (2-4 x 10^9 cpm/µg) by oligolabelling (Pharmacia) with ^{32}P dCTP (3000 Ci/mmole). Free nucleotides were removed by chromotography on sephadex G-50 (NICK column, Pharmacia), and the purified ^{32}P-labeled subtracted cDNA used to probe the subtracted cDNA libraries.

Example 3 - Screen subtracted libraries

The IEG-Reg and IEG-Lg cDNA libraries were plated on NZCYM agarose plates at a density of 500-800 plaques/plate. Duplicate nitrocellulose filter lifts were prepared from each plate using standard techniques. The filters were prehybridized overnight at 68°C in 5X SSPE (pH 7.4), 10% dextran sulfate, 0.2% SDS, 5X Denhardt's Solution, and 50 µg/ml boiled, sonicated salmon sperm DNA. The first lift from each plate was then hybridized with 4 x 10^6 cpm/ml of the control subtracted cDNA probe and the second lift with 4 x 10^6 cpm/ml of the 3 hour MECS stimulated subtracted cDNA probe. Hybridization was done in freshly prepared 5X SSPE (pH 7.4), 10% dextran sulfate, 0.2% SDS, 5X Denhardt's Solution, and 100 µg/ml boiled, sonicated salmon sperm DNA at 68°C for three days. Filters were washed twice at room temperature for 30 minutes in 2X SSC/0.2% SDS, twice at 60°C for two hours in 0.5X SSC/0.2% SDS, and then dried and exposed to X-Ray film for one to seven days. Clones

exhibiting greater hybridization signals with the stimulated cDNA probe than those observed with the control cDNA probe were picked for further analysis.

The putative neuronal IEGs were analyzed by reverse northern analysis and northern analysis to confirm that they were true differentially hybridizing cDNAs. The nucleotide sequence from the ends of these cDNAs was determined, and those sequences not matching the sequences of known genes were used to obtain full-length cDNAs from cDNA libraries.

Example 4 - Construction of a cDNA library enriched for near full-length IEG cDNAs

Since the initial isolates for all of the IEGs represented small cDNAs derived from the 3' regions of the corresponding RNA, it was necessary to rescreen other libraries to obtain full-length or near full-length cDNAs. For this purpose, a cDNA library enriched for neuronal IEGs with very long inserts was prepared from 3 hour MECS/CHX polyA$^+$ RNA isolated from rat hippocampi. This RNA was already relatively enriched for neuronal IEGs since the MECS/CHX stimulus produces a large induction of IEG expression. Further, the cDNA was synthesized in the presence of methylmercuric hydroxide to eliminate RNA secondary structure allowing for the synthesis of long cDNAs using Superscript II Reverse Transcriptase (BRL).

The basic protocol used to synthesize cDNA was as follows. First, RNA secondary structure was denatured with methylmercuric hydroxide which forms adducts with imino groups of uridine and guanosine in the RNA and disrupts Watson-Crick base pairing. Briefly, 22 µl polyA$^+$ RNA (0.5 µg/µl in either 10 mM Tris/1 mM EDTA (pH 7.0) or water) was incubate at 65°C for five minutes and then cooled to room temperature over five minutes. Once cooled, 2.2 µl 100 mM CH_3HgOH (90 µl depc'd water plus 10 µl 1 M CH_3HgOH) was added, and the mixture incubated at room temperature for one minute. After incubation, 4.4 µl 700 mM 2-mercaptoethanol (190 µl depc'd water plus 10 µl 14 M 2-mercaptoethanol) was added, and the mixture (final volume 28.6 µl) incubated at room temperature for five minutes.

Second, the first strand of cDNA was synthesized as follows. The volume of the denatured RNA mixture was adjusted by adding 26.4 µl water such that the concentration of RNA was 0.2 µg/µl. In the radioactive reaction, 5 µl (1 µg) polyA$^+$ RNA, 2 µl 10X Strand 1

Buffer (Stratagene), 1.2 µl Strand 1 dNTP mix (Stratagene), 0.8 µl Xho/dT linker primer (1.4 µg/µl), 5 µl water, 3 µl $dCTP^{32}$ 3000 Ci/mmole (NEN), and 1 µl RNase Block (Stratagene) was combined, and the mixture (final volume 18 µl) incubated at room temperature for ten minutes to allow the primer to anneal to the RNA. In the nonradioactive reaction, 25 µl (5 µg) $polyA^+$ RNA, 5 µl 10X Strand 1 Buffer (Stratagene), 3 µl Strand 1 dNTP mix (Stratagene), 2 µl Xho/dT linker primer (1.4 µg/µl), 9 µl water, and 1 µl RNase Block (Stratagene) was combined, and the mixture (final volume 45 µl) incubated at room temperature for ten minutes to allow the primer to anneal to the RNA. After the room temperature incubation, 2 µl and 5 µl of reverse transcriptase mix (4 µl Superscript II (BRL 200 U/µl) plus 1 µl MMLV RT (Stratagene)) was added to the radioactive and nonradioactive reactions, respectively. The reactions then were incubated at 40°C for one hour and placed on ice. Two µl of cDNA was removed from the radioactive reaction and added to 18 µl $T_{10}E_1$ and 2 µl 0.5M EDTA. Two (2) µl of this mixture then was applied to a PEI strip to determine the percent incorporation and quantity of cDNA synthesized, while 18 µl was mixed with sample buffer and ran on a gel to assay cDNA quality.

Third, the second strand of cDNA was synthesized as follows. Both the radioactive and nonradioactive reactions were kept on ice to prevent "snapback" cDNA synthesis. For the radioactive reaction (18 µl), 10 µl 10X Second Strand cDNA Buffer, 3 µl Second Strand dNTP mix, 62.5 µl water, 1 µl RNaseH (1.5 U/µl), and 5.5 µl DNA Polymerase I (9 U/µl) was added, and the mixture (100 µl final volume) incubated at 16°C for 2.5 hours. For the nonradioactive reaction (50 µl), 20 µl 10X Second Strand cDNA Buffer, 6 µl Second Strand dNTP mix, 111 µl water, 2 µl RNaseH (1.5 U/µl), and 11 µl DNA Polymerase I (9 U/µl) was added, and the mixture (200 µl final volume) incubated at 16°C for 2.5 hours. Four (4) µl of cDNA was removed from the radioactive reaction and added to 18 µl $T_{10}E_1$ and 2 µl 0.5M EDTA. Two µl of this mixture then was applied to a PEI strip to determine the percent incorporation and quantity of cDNA synthesized, while 18 µl was mixed with sample buffer and ran on a gel to assay cDNA quality.

The cDNA from both the radioactive and nonradioactive reactions were extracted twice with phenol/chloroform/isoamyl alcohol followed by one extraction with chloroform/isoamyl

alcohol. After extraction, the cDNA was precipitated with 100% ethanol, pelleted by centrifugation, and resuspended in 39.5 µl water. To blunt the cDNA ends, 5 µl 10X T4 DNA Polymerase Buffer (NEB), 2.5 µl dNTP mix (2.5 mM each dNTP), and 3 µl T4 DNA Polymerase (3 U/µl) was added to the 39.5 µl of cDNA, and the mixture (50 µl final volume) incubated at 16°C for 30 minutes. After incubation, 350 µl TE (pH 7.5) was added, and the mixture (400 µl final volume) extracted twice with phenol/chloroform/isoamyl alcohol followed by one extraction with chloroform/isoamyl alcohol. After extraction, the cDNA was precipitated with 100% ethanol, pelleted by centrifugation, and resuspended in 17 µl water.

EcoRI/NotI adaptors were ligated to the cDNA, allowing for the quick identification of artifactual cDNAs generated by the ligation of two independent cDNAs prior to ligation into the lambda phage vector. To ligate the EcoRI/NotI adaptors to the cDNA, 3 µl 10X Ligase Buffer, 4 µl EcoRI/NotI Adaptors (1 µg/µl), 3 µl 10 mM ATP, and 3 µl T4 DNA Ligase (400 U/µl) was added to the 17 µl cDNA, and the mixture (30 µl final volume) incubated at 10°C overnight. After the overnight incubation, 1 µl T4 DNA Ligase and 1 µl 10 mM ATP was added, and the mixture (32 µl final volume) again incubated at 10°C overnight. After this second overnight incubation, 270 µl TE (pH 7.5) was added and the mixture extracted twice with phenol/chloroform/isoamyl alcohol followed by one extraction with chloroform/isoamyl alcohol. After extraction, the cDNA was precipitated with 100% ethanol, pelleted by centrifugation, and resuspended in 30 µl water.

To kinase the cDNA ends, 4 µl 10X T4 Polynucleotide Kinase Buffer, 4 µl 10 mM ATP, and 2 µl T4 Polynucleotide Kinase (10 U/µl) was added to the 30 µl of cDNA, and the mixture (40 µl final volume) incubated at 37°C for 30 minutes. After incubation, 2 µl T4 Polynucleotide Kinase was added, and the mixture (42 µl final volume) incubated at 37°C for 30 minutes. After this second 30 minute incubation, 170 µl TE (pH 7.5) was added, and the mixture extracted twice with phenol/chloroform/isoamyl alcohol followed by one extraction with chloroform/isoamyl alcohol. After extraction, the cDNA was precipitated with 100% ethanol, pelleted by centrifugation, and resuspended in 85 µl water.

To digest the 3' cDNA ends with XhoI, 10 µl 10X NEB Buffer #2 and 5 µl XhoI (20 U/µl) was added to the 85 µl of cDNA, and the mixture (100 µl final volume) incubated at 37°C for 45 minutes. After incubation, 3 µl XhoI (40 U/µl) was added, and the mixture (103 µl final volume) again incubated at 37°C for 45 minutes. After this second incubation, 120 µl TE (pH 7.5) was added, and the mixture extracted twice with phenol/chloroform/isoamyl alcohol followed by one extraction with chloroform/isoamyl alcohol. After extraction, the cDNA was precipitated with 100% ethanol, pelleted by centrifugation, and resuspended in 20 µl 10 mM Tris (pH 7.5), 1 mM EDTA, 100 mM NaCl, and 5 µl loading buffer. This resuspended cDNA then was size-fractionated on a 1 ml BioGel A-50m column to select large cDNAs. The column was rinsed with 10 mM Tris (pH 7.5), 1 mM EDTA, and 100 mM NaCl. Thirty-six (36) fractions (50 µl/fraction) were collected. Aliquots from individual fractions were electrophoreses on 1% agarose to identify fractions containing cDNAs longer than 2 kilobases. Such fractions were pooled, and the resulting mixture of pooled fractions was extracted twice with phenol/chloroform/isoamyl alcohol followed by one extraction with chloroform/isoamyl alcohol. After extraction, the cDNA was precipitated by adding 2 µl glycogen (20 mg/ml) and 2 volumes 100% ethanol, pelleted by centrifugation, and resuspended in 5 µl water.

To directionally clone the cDNA into UniZAP, 2 µl UniZAP (500 ng/µl), 1 µl 10X T4 DNA Ligase Buffer, 1 µl 10 mM ATP, and 1 µl T4 DNA Ligase (4000 U/µl) was added to the 5 µl of cDNA, and the mixture (10 µl final volume) incubated at 12°C overnight. After incubation, the cDNA was packaged into phage particles. To package the cDNA, the ligation reaction (10 µl final volume) was divided into two packaging reactions with each containing 5 µl of ligation reaction together with a packaging extract (Stratagene). This mixture was incubated at 22°C for 2 hours. After incubation, the two reaction mixtures were pooled and the library titered on IL1-Blue MRF cells.

This 3 hr MECS/CHX library (designated IEG-FL 3 hr MECS/CHX cDNA library) had a titer of 4.4×10^6 primary phage. The library was amplified and used to isolate full length cDNAs derived from novel neuronal IEGs. The relative abundance of near full length neuronal IEG cDNAs in this library was substantially higher than the levels experienced using other cDNA

libraries. In a single cDNA library screen, full length cDNAs for four different novel IEGs were obtained. Three of the four IEG cDNAs were derived from mRNAs of 4 kilobases, while one was derived from an mRNA of 3 kilobases.

The nucleic acid sequencing of the IEG cDNAs was performed at Johns Hopkins School of Medicine and at Applied Biosciences, Inc., CA using the Sanger method with fluorescent dye termination.

Northern blot analysis was performed both to confirm that the cloned cDNAs represent tissue mRNA that is rapidly induced by brain activation and to assess the size of the mRNA transcript. The latter is essential information for the identification of authentic full length clones. Either 20-25 μg of total RNA or 2 μg of polyA$^+$ RNA was sized by denaturing agarose gel chromatography and transferred to nitrocellulose. Blots were then hybridized with [^{32}P]labeled cDNAs. Labelling was done using the random primer method (Pharmacia).

In addition, *in situ* hybridization was performed both to confirm that the cloned cDNAs represent tissue mRNA that is rapidly induced by brain activation and to confirm that the mRNA was induced in activated neurons. *In situ* hybridization was performed as described previously (Andreasson and Worley, *Neuroscience* 69: 781-796 (1995)).

Example 5 - IEG nucleic acid

The following clones were identified as being IEG nucleic acid as described in Example 3. In addition, certain clones were identified by chip-hybridization between PCR fragments generated from rat hippocampus ESTs and ^{32}P-dCTP-labeled cDNA derived from polyA$^+$ RNA of rat hippocampus from MECS treated animals and controls.

One IEG nucleic acid clone was designated A003. The first library screen produced a fragment (A003-1-1) of 1.6 kilobases (kb) with a polyA sequence at the 3'-end. A second round of screening was performed using a probe prepared from the 5'-end of A003-1-1. This screen produced two clones: A003-1 (2.8 kb) and A003-2 (1.3 kb). The fragments from the secondary screen were sequenced from both ends. These fragments formed a contig at their 3'-end with the A00-3-1-1 fragment. The following two nucleic acid sequences are within the A003 clone: 5'-

TTGCAGATCAGCACCTTTTGATGATGCCTGCCCAACAGTGGGTAATGCTNACAGCAA
AGCACCACTTTACGCTTTTTAGTTGTGCTGGGTTCATGGCTGGACATACACCAACCA
GCCTTGACCCCACAGGAATGCCAAGTTGGCTGGAATGTAACCCAACCTAGTTTCTGC
GCTTCGCTCCTCTCCCAGTGCAAGGTGCTAAACACCCACTCACAAGCCTGCTGTCAA
GCTGCGACCTTGGGGGCTGGTTAGAAAGGGCTGCCTCCTTCCAGCAATAGAAGTTCA
TGAATTTGAGGCTGGAGATAGGTCAAGACCACTGTGATAACTATAAAGACTGTAGC
AGCCACAAAGGAGACCCCCAAATAACTGGAGGCATGGGCACTGACGTACCAGATGA
GGTTATGTTTGGAGCTGAAGGCTTGCTCTGTGCTTCTTGGTAGCATCTTTTGTCCTCT
TGGGACATGGTTGACCCCATACTGTCCACTGAGCTTGGGAGATGACAGTTGAATAAA
AAAAAAAAAAAAAAAA-3' (SEQ ID NO:1) and 5'-CGGCTTAATTAACCCTCACTAAA
GGGAACAAAAGCTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCC
CCCGGGCTGCAGGATTCTGCGGCCGATTAAGAAGCCTGCTGATGTCCTTAGGCGAGG
ACATTAACTCCAGTCTCTGACAGACTTTGGACATCCAGAATAAGTTCTTTTTGTATAT
CAGAGCACAGAGCCCAGCTTTAGCCTCTGATGGACCTCAGGAACCAAGAAGGAGGG
ACTTCCTTAACATTCTAGAGATGGGACTCTAACTCTAGCTCTTGTGTTAAGCCCTGAA
GTCCAGAAAGAAGTAGTTCTTTGACATTCTAGTGCCAAGATCCAGCCTCTAAGAGAA
CTCTGATGTCTAAAGAAAGTCTTTCATAGTCTAGNCCAGTCACCAGTGAAGCTAAAC
ACCTGAAAACTATTAGATTCTCTGGAGCCAGGAATCCATCTCAAGTCTCTCATAAAG
CCCAAATGTCCCAGGAGAAGTTGACAATATAAAGCCGTATCTCGATGGACTTTTGAA
GAAGCTCAGAAAAGGAGACCACCTTGGTAGTCTTGATCTAGGACTCTGGCTTGTTTG
TCTCCAGGGACGTTTACATGTATAAAAAGAGGGACCTTTCTGATGATTCAGAACTGG
GACTCCACCTCCATCCTTTGATGAAAGCTCAAATGTCCAGAAAGAGGGGCCTCTCTG
ATATTCTAGAGTAGGACCCTCCCTCCAGCCTTTGATGGTGTCCAGATGTCCAGAAAG
AGGGGCCTCTCTGATGTTCCAGACCTAGGGCCCTCCCTCCAGCCTTTGATGGTGTCC
AGATGTCCAGAAAGAGGGACTTCTCTGATGTTCCAGACCTAAGACTCTAGCTCCAGC
CTTTGATGAAGCTCAGATGTTCAGAAAGGGGGGCCTCCATGATGTTCTAGAACCAGG
ACTCCACCTCTAGCCTTTGATGGTGTCCAGATGTCCAGAAAGAGGGTCTTCCATGAT
TTCTAGGACCAAGACTTTACCTCCAGCCTTCTATGCCTCCATGTCTCCAGTAAAGCTT

AGGTGTCCAGAAAAGAGCATTCTCAATGAATTTATAGAACCAGGACTCTTTCTCCAG
CCTTTGATGACGTTCAGATGTTCATAAAGAAGAACTTCCACAATGTACTAAAGCTAT
GACTCCATCTCCATCCTTTGATGAAAAGGGACTTCCTTCCACTCTGTTCCAGAAGCCT
AGCTCCACCTCTAATCTTTGTTGATGTCCAATTATCCAGAAAGAGGGGGCCTTTAGA
ACAAAGACTGTACTTTTATTCATTGATAAAGCACAGATTCCAGAAGCACAGAAATCT
AGAAAGAGGGTCCTCCCTAACACGCTCGAGCTAGAACCCCGGTGCAAGGGTCTGAA
ACTTAGACACCAGAAGACCGCTTTGTCCTACAACAAGTCTGCATTTTCTAAATCTCC
AGGTGGCTGAT:CAGAAGGGTCCAGGAAGGTATGGGG-3' (SEQ ID NO:2).

Northern blot analysis using the 3'-end of A003 revealed the presence of two mRNA transcripts. The more abundant transcript was 2.2 kilobases in length, while the less abundant transcript was 4.8 kilobases in length. This analysis also revealed that the expression of A003 mRNA was marginally upregulated in response to the multiple MECS treatment. The multiple MECS treatment involved the induction of multiple maximal electroconvulsive seizures followed by the preparation of total RNA from rat hippocampus four hours post-seizure. This multiple MECS treatment was designed to mimic ischemia.

Another IEG nucleic acid clone was designated A013. The first library screen produced the clone designated A013-8. The 5'-end of A013-8 was used as a probe for the second round of screening. This second screening produced two additional clones: A013-4 and A013-26. The A013-8, A013-4, and A013-26 clones were sequenced using either the gene specific primer used to generate the probe for the second round of library screening, or the T3 and T7 primers. Both A013-4 and A013-26 made a contig on their 3'-ends with the A-013-8 clone. In addition, the sequence from the 5'-ends of A013-4 and A013-26 revealed that they from contigs between each other. Further, the sequence data from the 5'-ends of A013-4 and A013-26 revealed the presence of an open reading frame of at least 720 basepairs (bp) Based on the combined length of the obtained clones, the A013 clone is at least 3.0 kb in length. The following two nucleic acid sequences are within the A013 clone: 5'-GGCACGAGATCACTCAGTGTCTTCACTGAAC
CAAATCGTCATTTTTACAGAGAGATGCAAAGCTTCAGCGAAGACATTTAGCTTTTTT
AAAATGTATAATTCCTGTGGCTACATATGCAAGTAGGGTCCCATTATGTTTTTTTTCA

TTAGTGGAAACTAATCCTTTTGTGCTGTGTTTAATCAGTATTAGCTTTATAGAATTAT
AAATGTATATTCTACTTCTTGATCAAAGAACGTAGTCGGGTATTGGTTTTAGAAGTTC
AAAGTGACACTGTATAGGGCTTTCACGGTTAATGGGATTGTTAGCAAATCTTAAGGA
CATACAGCCAATGATTATCTGAGGTTACTGGCTAACTGTTTTTCACTGAGTTACTCTG
CCTTTTTGACATTTTTATTCTTTGTTTGTCAGAATCCAGAGCTTCAGGAGCCCAAATT
TTTTTATWCCGTATATATATATATATATAAATATCCATAAGCCTGGTGGATTTGTATG
CAATGCACTGCATCTATGTATTCTGATAGCATCTCATTGATTTTTGTTTGAAATAGAA
AGAAAGATAGTATCCCAAATGAGTTATCTTTAACAGAAAGCTGAGTTTAACTTTTAT
TACCTATATAATAATTGATATTGCCAATTACCATTCTGAATTTCATATAGTATAAGTT
AGACATTGCTTAATCCCCTTTTAAATGTATTTACATAGACATGAACACTCAAATTGCT
GGATTTTTTAAATATATCTGACATAATTTTTTTCATCTGTTACATTCAAGTTAGCTTGT
TTAGCCCAGATTTCAGAATAGTAAAGGAGGAAAGGAACCGCATTCCAGGGAAACCT
CTGAGGCCAAGTCAGAGTCCAGAACTGTAAACACACAGGCCTGCAAGCCAACATTA
GTCGTGAAATCCCTAACACGTCACTGGATTCTCTCTGTCAGCGCAAGTGTCAGCTGC
CAAAGAATAGACTTACATGAAGAAGTGCCCACATGCTGGCAGGGGCTGGCCGGCTC
CGGCCAGCAGACACTGCTAGATTGTAATATTTAAGGTCGAGTTTCGACCTGTGGTAC
ACAGCTGTGCTGTGCTCAGTCAGCAACCTCAGAACTCTGAAAAAAACATAAAAAAG
AAAAAAAAAAAAAAAAAAAMATGCASCTGKYTCACTTGTGAATAGTGAATGTAAAG
GAAAGAAAGGAAAACCAAAAGCTTGTTCCATCACAGGTATGAGCTGCTATGATTCA
TGAAGAACATTCCATGGAGTATGTTTTAAAACCTTGTTATATCTGAGAGGCTTTAAA
AGCCAACTTAACTGTTTCAGGGCAACCGCGGTACAGACGTGGTCTCTGTGAGACTTC
CACCTGACCCAAGTTTTAAGTGGTACGAATGTTGTGCATTTAATGTTAAGGACAGTC
TGCAATAATAAGTAAGTAGCCAGCGTGGGTGCCCAGCAGTGCTGAGACCTGGCTGC
TCTATTGTACGCTTTGGAAACACAATTTATGCAACAGATGTCCAGATATGATTCTATT
TATGGAAAAAGTTTATATGTTTTACAAATGGTTTTACCATCTTATATTAAATGACCTT
TTGACAGGTGTGCACTGTTTTGTCTCCAGTGAGCACATACCATGCGGATTTTATATGT
ACATCAGTAGTGTGAATCCACTGGCACAGTGTGTGTAAATGCCAGATGTGGTGAGAT
TTTATCTTGTATATGTGATCAGATAAAATAACTCCTGACAGAAACTGTAAGGRAACC

CAGCTGAATGGTTTGACCTGGATGRCYKRKRTKGTWTGGTTTATGTTAAATGTATAT
TCTTTTAATCAATGAATAAAGCATTAAAAAATGGGAAAAAAAAAAACTCGTGC-3'
(SEQ ID NO:3) and 5'-TCTGCGGCCGCAGCATCCGGAACAACAGGAACCTCCAGAA
GTTTAGTCTTTTTGGAGATATAAGTGTCGTTCAGCAGCAAGGAAGTCTGTCCAGCAC
ATACCTCAGCAGAGTAGACCCTGACGGCAAGAAGATTAAGCAAATTCAGCAGCTGT
TTGAAGAGATACTGAGCAATAGTAGGCAACTAAAATGGCTGTCCTGTGGGTTTATGC
TGGAAATAGTAACCCCATCATCACTGTCGTCTCTGTCTAACTCCATTGCCAACACCAT
GGAACACCTGAGTTTACTGGACAACAACATTCCTGGTAACAGCACGCTCATCACCGC
AGTCGAACTAGAGCGCTTTGTAAATCTGCGCTCACTTGCCCTGGATTTCTGTGACTTT
ACAGCTGAGATGGCGAGAGTCCTGACCGACAGCAACCATGTGCCTTTGCAGCGACT
GTCTCTTCTGGTCCACAATGCTTCAGTGATGCTCAAGTCATTAGACAACATGCCAAA
CGATGAGCACTGGAAGGCCCTGTCACGAAAGAGCTCCAGCCTCCGGGTCTATCTAAT
GGCTTTTGATGTTAAAAGTGAAGACATGCTAAAGATTCTGAAACCCAGTATACCACT
TGAGAAGGGTTCACTTTGGACAGCTACGTCACTTGTGTCTCAAGGGGCTATTGGTTG
ATCTTATATTCCAGGCAGTATTGACCAAGGTTTCCTYAACCCMWTTTWTATTGATGA
ATGATATGATTGATACGTCTGGTTTTCCGGATCTTAGTGACAACCGAAATGAAGATC
CATTGGTTTTATTGGCATGGCGGTGCACAAAGCTCACTCTTTTGGCAATTCATGGTTA
CACCGTGTGGGCACACAACCTCATTGCCATTGCTCGTCTTCGTGGCTYTTGACCTAA
AAGTGCTTTGGAAGTCACCSRAAGAAAGCATTGATTTTGACCAAGGTGAACTAGCCC
GACCAGGAATGTGGRWYCCCGTACATAACCTTTCTTGGAGCAGGTATTCCCTGGGGC
CTTGGTCAAGTCTTGGCACG-3' (SEQ ID NO:4).

Northern blot analysis using a sequence from the A013 clone revealed the presence of a 3.2 kb mRNA transcript. In addition, this analysis revealed that the expression of the A013 mRNA was strongly upregulated in response to the multiple MECS treatment. Specifically, A013 mRNA expression was induced 8.9 fold by the multiple MECS treatment as determined from Northern blot data using total RNA from rat hippocampus (Table I).

Table I. Fold induction of mRNA expression after multiple MECS treatment

Probe (rat cDNA)	Fold induction (normalized for the S26)
A013	8.9
L094	7.3
L100	17.2
L119	17.8
R113	7.0
R286	2.4

Another IEG nucleic acid clone was designated A020. The following nucleic acid sequence is within the A020 clone: 5'-TCAAACCNTATCTCGGTCATTCNTTTGAT
TNATAAGGGATTTKSCCGATKTCCGGCNTATTGGTTAAAAAWTGAGCTGATTTAACA
AAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTGCCATTCGCCATT
CAGGCTGCGCAAYTGTTGGGAAGGGCNATCGGTGCGGGCCTCTTCGCTATTACGCCA
GCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTT
CCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTGTAATACGACTCACTATAGG
GCGAATTGGGTACCGGGCCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGATATCG
AATTCGGCACGAGCGAAGCCAGGGCCTTGCACTTCCTAGGCAAGCGCTCTACCACTG
AGCTAAATCCCCAACCCCTTGTTTTATTTTTAAAGCAAACGAGATACATAATTTCARC
CATGATAATTTAAGATTATCTTGAACTCTTAAGGAAATGTATATACTAAGCTATTAT
AGTTTTTATTTTCCCTAATTCAGTGGCATAATACCTTACCTTGAGTCGTTTACTACTTT
CTTTGGTTTCTAAAAACTCTACTGCTAAATTACAATGTAAAAACATAGGGCTCGTAT
ATACTGTAGAGTGCTGTAGATGTCCTCGTCATCAACTATGCAATAACAGTCTGATCG
ACACATTTCAGGAKCGATCACTCTTTGGTGTGCTTCTTTAAATACTTTCAGAAGCTTA
GGATGTGCAAAGCAGGAAGACTGTGGGTGTAAATGTTTACTTATTTCTTTGAGAGTG
TTAGTAAGTCTTTTCDAAATTGCTTTTCTCTTCAAAATTATCGTTAACTTAAATGATA
ATTATCTTTGAGGTTAAACAGAAGCTCATTGACAAACTAAAGTGACTTTTTAGGGCA
TTCTTTGAGATCATAGTCTTATATCTTGGGGACTAAAATGTCATTAGACCCTAATAGA

CTAACTTGTATGTTTGTGTGGGGAAACGTTTTCCTCTCTCATTCAAGGTAACTGTTTG
CTGCCTGTTGTTACTTGTGTAGCATTCTAGAAAATGGCTAGGTTTTTTATAAGATTTA
AGACAATAGAAGTAGTTTTATATTATTATAGTTCTGTTGGAATGTGATCCTGAAATT
ATTACTGAAAATTAGAATTTTTATTTCGCTAATGACAACCTTGACTCTCAGAGATGC
AGTGTAAATTGATACCTCATCTTTCCGAGAGTTCAGAGCACAGGGCGGCAGTATGTG
AAGCTGCTTTTGCACTGACGCATTTTGATAAGTTTGGCTACTGTAATGGTAAAAGGC
TCCTCAGGCACTGACTGCATTTGGGTTCTTCCGATGGGGGATGATCCGTTCTCGTGGT
GCTGCTGGACTTATGCATTTTGGAGGTACTGCATGTATCTTCCACACTGCTTGACATT
TTCTCTGATCTGTGTGTTTGCACCAACTCATTAAAAGAAATATGCAGAAATATCTTCT
AATTCGTTGATCTTCGCTGTATGACAGTTATAATATTAAACACTTGGGTTGATCCACT
CTGTTTACATTTATCTTTCTAAGCGTCAGAAAGGGACTAACTTGAAATTATATCTAGA
GGCTTTGTATCATTTCAAAAATTAAATTTCCTTGGATACTTTAGGCAATATCTTAAAC
AACTTTTTAATAAATTTAAATATTTATATTTACGTAAGCTAAAATATACATGAATGTG
CTTTTTAATAAATTAAATACAGTTTATACTTATTTGCCAATTCACAAATAAAAAAAA
AAAAAAAAAAAAAAAA-3' (SEQ ID NO:5). This clone is similar to GLGF-domain protein Homer (accession # U92079).

Another IEG nucleic acid clone was designated A021. The following nucleic acid sequence is within the A021 clone: 5'-TTTTTTTTTTTTTTTTTTTTAARGGGRCCACCCC
ACCGSGCTAAAGGCCCAGGGGCCCCCCCCTTGGAGMCCCAGGGGTTTTGGCCCMCC
CCCTCACCCAAATGGTCTGCCAATGACCCAGGTACTCACAACATGTTCCAGGAGGAG
MCTGGGGCCAGGATTTTGACCAGAGGGTATGGGAAGGGAAAGGGGAGAAGAAATC
GACATTTATTTTTATTATTTATTTTAAATGTTTACAWTTTCTTTGTGTTGTTCCAAGCC
CTGAATAGAAACAGATAGCATTAAAGGACTCTGTTCCCACCCCTTCTCTGTCTCTCTC
TCCCCCACTTGTGCTAACTTAGGATAACACTCTCTATTTCGTTTTGTTTCTAAAGTGA
TTTGTGGACTTGTGCCGTGTGAACTGCATTAAAAAGGTTCTGTTTTCAAAGATCGATT
GTCGTTCCTGTGGGGACAGTGGCTCCTAAGAAATCTGCATTGTAGGAGAAGACAATG
AAAGACCCTGGCCCTGTCTCTCAAAACTTAACTCTCTGTATGATTTAAAAAAAAATT
CCATTTACTTTACTTTGTGGTTACTTGATTTTGAGGAAGAAAATATTCAACTTTGTAT

AAAGACTAGGTATCAGGGTTTCTTTTGCAGTGGGAGTTGTATATATATCGTATTTTGG
TATATCGTAGAAACTCAAGCTTTATGCATCCGTATTTGGGATATGTCAATGACGTGC
AGTGAAATTTGCTATTAGACCCTGGAGGCAAACGAGTTGTACAAGGTTTTATGGCTC
CATGGGGAATTCTAATTTCCTTTCTGGGGACCTTTTGTCCCGTTTTTACAGTAATGGT
GAAATGGTCCTAGGAGGGTCTCTCTAGTCGAATTCTCCAGGCAGGACCACGTGCTCA
AAAAATCTTTGTATAGTTTTAAATTTTTGAGGAGTATCTCTGCTCAGAAGCATCTGTG
GTGGTGTGTGTTGCGTTGTTCTGTGTACTGTGTGTGACACAAGCCTACAGTATTTGCA
CTAAGGAAAGCTGTTTAGAGCTTGCTGCTATGGAGGGAAGAACATATTAAAACTTAT
TTTCCCTCGGGGWTTRTWCWMGTTTTATGTWCTTGTTGTCTTGTTGGCTTTCCTACT
TTCCACTGAGTAGCATTTTGTAGAATAAAATGAATTAAGATCAGMWRWRWRMAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-
3' (SEQ ID NO:6). This clone is similar to fra2.

Another IEG nucleic acid clone was designated A024. The following two nucleic acid sequences are within the A024 clone: 5'-TCAGGCCTNAGCAATCCTCNTTAANTTTGA
NCCAAGNTTAACTCTTGGGGCGAATTCCTGTGNTTGCTTTCTTTCCCCATANTTCCAG
GCCCACAAANGGTTTCTGTGANTCCGAGAATCGGCCCACCATGCAGACCCACNGAG
AGGATTCAGAATGTGTGTGAGAGTGAGTGTGTGAGTGCGCGTGCGTGTGCTTTGTAT
GTGTGTTTATAGATGTAGGACATTAAGTTCCTTCTGACACAGGGAAGATGTGAGAAG
GATGGCCTGACATCAGATGACAAGAGGTCTTATAGCACATCTCTGGGCTTTTCCCTA
CCCAGAGAAGAGCCCCCTTTGATACAAATCAGTTGGATTTTCATATGCTTCAAAGGC
TTGATCTGTGAGTCACTCCAGTTTGGGACATAGGTCTGTCTGTGGCTTTGAGAAAAG
GTACTTTCAAAAGAGGGCTTTCCAGAGCACAGCTCACAGCCAGCTGTTAGGACCCCA
CCCTTCTCCTTTATTGTGGAGGTGACTCACAGCAGACTGACAGTGGTCAGACTGAGC
TTTCTGCTAAGGTGGTGAGGTAGCCAACACTGGCATGTCTCGGTAGTGGTTTGGGCA
AATTTCCGCAGGTCTCTTCCCCCAACCCTGCCTCTGATGAATAAAGACAATGAGTAC
AGTTCCTTAATTCAGGCTTTTGTGACTAGCTTACTACGGAACCGAAAATGGTCCCCTT
TGTACAAGCCGAGCTGTTATGGAATCACGGTGAACCAGACCCAGGTCTGTGGCACCT
GTTTGTTTTTTTTTTTTTTTTTTTTTTTTTTAGCTCTCATTTCTACGGCATGCTTTCCAAG

GAACCAAAGGAGGGTCTCAGAGATGCCCCAAACATCCCAAAGTACACAAAGCTAAG
TAATCGATTGCTTACTTATTGCACAGCTAGACACGGATTTTAAGTCTATCTTAAAGCT
TTGAAGCAAGCTTAGCTTCTCAAAGGCCTAGCAGAGCCTTGGCACCCCAGGATCCTT
TCTGTAGGCTAATTCCTCTTATCCAGCGGCATATGGAGTATCCTTATTGCTAAAGAG
GATTCTGGCTCCTTTAAGGAAGTTTGATTTCTGATTCAGAGTCCTTGTTTCCCTGACT
TGCTCTGCCAGCCCTGCACCAGCTTTTTCGAAGTGCACTATGCTTGTGTTTAACTTCT
CCCAGTTTTATTTGGGCATAAAAGTTGTTGCCTTTATTTGTAAAGCTGTTATAAATAT
ATATTATATAAATATATGACAAAGGAAAATGTTTCAGATGTCTATTTGTATAATTAC
TTGATCTACACAGTGAGGAAAAAAATGAATGTATTTCTGTTTTTGAAGAGAATAATT
TTTTTCTCTAGGGAGAGGAGAGGTTACAGTGTTTATATTTTGAAACCTTCCTGAAGGT
GTGAAATTGTAAATATTTTTATCTAAGTAAATGTTAAGTAGTTGTTTTAAAAAGACTT
AATAAAATAAGCTTTTTCCTGTGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3'
(SEQ ID NO:7) and 5'-GTGGCCCCTGCTCGCCGCATCATGGAGCGGATCCCCAGCG
CGCAACCACCTCCTACCTGCCTGCCCAAAACGCCAGGGCTGGAGCACGGAGACCTG
TCAGGGATGGATTTTGCCCACATGTACCAAGTGTACAAGTCCAGGCGGGGAATAAA
ACGGAGCGAGGACAGCAAGGAAACTTACAAATTGCCGCACCGGTTGATTGAGAAAA
AGAGACGTGACCGGATTAACGAGTGCATTGCCCAGCTGAAGGATCTCCTACCCGAA
CATCTCAAACTTACTACTTTGGGTCACTTGGAGAAAGCAGTGGTTCTCGAGCTGACG
CTGAAGCACGTGAAAGCATTGACAAACCTAATTGATCAGCAGCAGCAGAAAATCAT
GGCCCTGCAGAGCGGTTTACAAGCTGGTGATCTGTCGGGAAGAAATATTGAGGCAG
GACAAGAAATGTTCTGCTCCGGTTTCCAGACCTGTGCCCGGGAGGTACTTCAGTACC
TGGCCAAGCATGAAAACACTAGGGACCTGAAGTCTTCCCAGCTTGTCACTCATCTCC
ACCGTGTGGTCTCTGAACTCCTGCAGGGTAGTGCTTCCAGGAAACCATTGGACTCAG
CTCCCAAACCCGTGGACTTCAAAGAGAAGCCCAGCTTCCTAGCCAAGGGATCAGAA
GGCCCTGGGAAAAACTGTGTGCCAGTCATCCAGAGGACTTTTGCTCCCTCGGGCGGG
GAGCAGAGTGGTAGTGACACGGACACAGACAGTGGCTACGGAGGCGAATTGGAGA
AGGGTGACTTGCGCAGTGAGCAACCCTACTTCAAGAGCGATCACGGACGCAGGTTC
ACCGTGGGAGAACGCGTCAGCACAATTAAGCAAGAATCTGAAGAGCCCCCCACCAA

AAAGAGCCGAATGCAGCTCTCAGATGAGGAAGGCCACTTCGTGGGCAGTGACCTGA
TGGGTTCCCCATTTCTTGGGCCTCACCCACATCAGCCTCCCTTTTGCCTGCCCTTCTAT
CTCATCCCACCATCGGCCACTGCCTATCTGCCTATGCTGGAGAAATGCTGGTATCCG
ACCTCTGTGCCACTGTTATACCCAAGCCTCAACACCTCAGCAGCAGCCCTCTCCAGC
TTCATGAACCAGACAAGATCCAACTCCCTTGCTCTGCCCAGAAATCCCTTCTCCCTTG
GCACATTCGTCCCTTGACTCTCAAGCCTGCTCAAGCCCTGAAGCAGATCCCTCCTTA
AACTTAGAAACAAAGATAAACCTTGAGGGCAATCNCTGCGCCTTGCTTTCCTTCCCA
CAATTCAAGACACAAAAGGTCTGTACTCAAAACAGAGAGATCAGCCCACCCTGCAG
ACCCACAGAGAAGATTCAGAGTGTGTGTGAGAGTGAGTGAGTGTGCGTGCGTGCGT
GCTTGTATGTATGTTTGTATATGTAGGACAATAAGTTCCTTCTGACACAAGGGAGAC
ACGAGAAGGATAGCCTGACATCAGATGACAGACTGGAGGACTGTAGCACATCTCTG
GGCGTTTCCCTACCCAGAGAAGAGCC-3' (SEQ ID NO:8). This clone is similar to a basic helix-loop-helix polypeptide.

Another IEG nucleic acid clone was designated L003. The following nucleic acid sequence is within the L003 clone: 5'-GCACGAGGGAGTTTATTTCCACGTCT
CTTAGGAAAGCCTCGCTTGGTTACACATGGCAATGATTGCAAGCAGATACACGTCTT
AACACCAGAGTACAGTACACACACATTGAGCTGCCCTCGTCTAACAAGCAGTTGCA
GTTTGTTTAAATGTGAATATCTATGAAACGAGCAAAGCAACTTTCCAGAGTATAGCT
TATCACAGAATAGTAACACATGGGCCGCTACTGTATCATACAGAGTACAACTCTATA
GCTTTTCATCCCCGTGTGAGCATTTCCAAATCACTCAATGAGCACCAAGCACGGACA
AGTGACTAAAAAGGCTAGTCCCAATCTCCCCGCAACCCTCGGCGGTAAGGGTAAAG
AATTTTGTTTCAAGTAAGTTTTCTCCTCGTCTCTCTCTTCTGAAGACCTGAGCAAAAC
CAACATTCTAAACCACCCCAAGATATGATACTAGAATTTAAAGGCCCGATGGCTTCA
ACCCAGAACCTTAACCTACTAGATAAAATCTCTCCGAATCTGACTCACTGATGCTGT
TAAGTCCGACAGTACAATCACATAGTACCTCTTTGATACTGTCAAAGTTGGTTTTAA
AAATGCCCTAAGAAAACCAAATCATTTTTGGGAGATGTTCTAAGCAAGCTTTCCAAC
ATATAAAGAACAAAACCATGTTACTAAAAACATGGTGCAGGTCCTCACAAAACATT
TACTGCTACTACCAGGAAACCAAGCTACTCTTGGTTTGTGCTCCTGGTGATAACTGG

TGAGCTTTGGACAGCTGCTGGCACATGTCCACTGTGTTCCGTTTTATAATCAAGTGTC
AGTTTTCCACTCGACAGAGATTAAAGACAATAGCTTAAAAGTGAAAATGAAATTTCA
AGTAGAAGCTACAATTGAATGCTACTTGTTGAGACTTTTAACTTTCACATCCAAATA
TCAAAAACTTAACTTTGACGACACATGCACACAAACACACCATTTGGGAAAGGGTCT
TGTTATGCAGTTCAAGCTGGCCTTGAACTCATGATCTCCTGCCTCAGTTTCTTGGGCA
GTAGCACTGGACCTTACTGTGGGCAGAAAGTATTGCTCCAATTAGAAAGCATTACTA
TACACTTCACTTCGTCATGTGCCTAGTGTGGCTCTGAAGGCATAGGAACAATGAAAT
TAAATTCTTCAGCAGCTGAGGATTCTCTATACTTCAACATTCTGAACTTCAATCATGG
CTTCACATTTGAGGCTGAGCTAGATACAAAAATATCAAAACATCCCATAGAATTGTT
TATTTCCCTATGTTACTGTTTACCCAAGGAATGTGAAGACTAAAAAGGACTCATTTG
GTTGTTTAATTATGATTAAATTATGTAAATATACAAACATTTAACAAAGCCATCATA
TTCCAATCTTTTACGAATTCTAACTGCTAGCAGTTGAGCAGCTTTTAGATATCACTAA
TAAAATATACAATTTAAAATAGTCGCATTCAATCCTACTAACTTTATAAATAACTTCT
TAGGTTAGACTTCTTCCTGCCTAAGTTTATAAGACAGTCTAAACCCAAAACTCAACA
CATATTAAGCTTTTTAAAAACTCCATATAGTTCTAAAGTAACCTCAATGTATTCCCAA
GAACCGCCACCATCAATCAGCTCACTCCCTCACACCACTGACTTTAAGACGCTCCTG
GGTGGAGAACTGCCAGGCAGAAGCTCTACCTTTCTAGTGTGTGTGGTGGTCTGCTGC
TCCTAGTCCAGATCTGGACCACATCAGCACAGCATCAGTGTGACTCAGCACTGAGGC
CTTGAGCGCTCTTCCCCCCGATGGCCTGTGTATAGAGGTGTCTAATTCCTTGTGTATA
GATGGCCTGTATATAGAGGTGTCTAATTCCTTGGCTCTGTATGTATAGGTAATGTGAT
ACTTTACCATTAAAGCACTATTTTCTCCATTCAAGAATTTAGTGATATAGGAAAATG
AGTGGACTTGCGAGACTCAGAAAAACAAAACATAACCTGTCTTGAATTCAAAACAA
ACCATGGGTGTAGGGGGGAACTGATGAAAGTTTATGGGTTTAACTCTAGGTAATTAA
CTAAGACAGTCACGAAACACATTATCAAAATCCTTTCAGGCCCAGAGCTTGTACTGT
ACCCCACTGTGAGACCACATCACAACCCCGGATTGAGCTTTATCCACAACACCTACA
CCATAGTAACGCAAAGTGCACAATGTACTAAAATAAATTCCTATTAGTTTTATGCAA
ACTATGGTATAAAATTATCACCTGCCATACATATTTTGCCATGGCACCAACTTCATAT
AATAAGCCAACGTATAATCAAAGTCCTTACCAGCACCAATCAATGTCCTTGGCACCA

CTGGACACTCACCGTCAAGCTGTTCATCTAAGAGCCAGTCTGTTCTGACCTGAACAG
TTGTGCATTCCACCTTACCACACCCAAGTCTGTGAGCCGGACAAGTGTTTAAATGCA
GTTTTACATCTAACGGTGCAGGTTAAGCCGAGCACTTGAAACTGATCACTCATTAAT
ACCTGTCTCCCTCCATACATGTACACCACATGTACACAGAACTATGTGCTCTGACTTC
AGAATAGCTCTTCCTGTTGGCAAAACACCACAGACATGAAGGGGCCTAGTGTGAAG
CGAGCTCACAGAATGTTGGATGGAACTTCGACTATAATGGAAACACCTGCAAAAGC
TTTGCTAACCCAGCAAACACTCAACACTTACCAAAGACAACAGGGAAGTTAAAGTT
AGCTCGCCAAGAGATGGGCTGGGGAGGTGGGGGTGTAACTCAAAGAAAGCTTTAGC
TAACAAAAACGAATGATGGACAACTTCAGAAATTCCCTAAAAACAGAACCTGAAAG
TGCAGGTGAGGTTTTGTCCTTCAGTAACAAATGCAGACAGATTCCCAACAGGAATAA
AACAGTCTGGGGCTTTGAAACCTGCTAGATGGAAACACGAACTCAAAATGTGGAAC
CAAGGAAAACCAAATACTTAAATGTGTAAGATAATTTATAATAGTAAAAAGTTGCA
AATTGCTGTGACTTGATTTGCCGAAAACATCTGTAAATCCACACTGGCAGTTAGAAG
ACCAGTTCCCACATTAACTCCTCTCTCAGCAGGTAACCGTTTGTGCGCAGAAGTATC
TGAAACATCGCACTACTGCTTATTTTATGGTGTATTGTGCAGAATCTGTACATGCTAT
TACAGACAATACATATTTGTAAACCTGGTCATGCAAAATCAGTGTGTACAAGGGGAT
ATTGTTAAGCCTTATAAAGTGGTACTTTATTATCTTTGTGACGATGCCAATCTCTCCG
AAATATAGCATATCTTAAATGGATATTCTTTATCTGCCAGTTAAAATCATTTTATGTC
ACTGAAAGAAGAGGTTATACAAGGAAAGAAACATGGTCCTTGTGTTGCAGAATTGA
TTTTAAATGAGAGAATTTACAAAACCAAGAAATCCATGGTCATAAAGTTTTAACATT
TTAATCCTACACATTACAGGGCAAACAGATACTGGACCCTATTTCCACATTCCATAA
ATCCAAACTTTAGTTCCCATTTCAAACGTTGCCCTAACCACTAAAACCATCAGTGGT
CTTACAACCTCTGGATTATGGAAATACAGATTTCTGAAGTAAAAGCTACAAAAACAA
CAATGGAAGAAAGCTGAACAAACTTCCCATGAATGAAAATAAAAGTGGAACATCCT
GAAGCTCTAGACACTTCTCTCCCGTGTCTATGGTCAACTTGTCGGTTCAGTGCACTGT
GCGGTCAAATGTAATGGTCCTCATGTGGAACACACGTCTAACTAGTGTCCATTGATT
CCAAGTTAGTGGACGAAGAATCTTTCTGGATACTTTCAAAGATGGCTGCCAGCTCCG
GGTTGGAGCTGATCTGTGACTGGAACTCACTCATGAGAGGGCTCTTCTCTGCCTCTG

GAATGGTGAGCAGTGCAGCTACTGCCCTCATGGCCGAGCGCTTTAACTCGTCCTGCT
TTTCAAACTCCTGCTTTACAGAGTTCGCCTTCACCTTAGTTGTACACGTAGCTCGTAG
TGGCTCAACAAGCCGGTCCAACCTCTGTAGTACTGCACTTGGACAAAGGGTAGATAG
TCTCACCAACATTAAAAATGTTAGCATCTTAATATCATAATGGTCCTTCAAACCATCT
TCCACATGATTTAGAAATTCAAAGATATCCAGTCTGTCAAGACAGCTGTCTAGAAGT
GTGTACATACACTCAAAAGCTGCCTTTCTAATGTCCAGGCCGTCATCAACCGTGTGC
TTAAACGGGCCCATCTCTACCTCTCTTATAAGTTCCTTCCTAACTTTTGTCTCATTGTA
AAGATGTGGAAGAACAGAATCCAGAAGGTCCCGTATCAGTGACGGCTTGTTATGGG
CTGCAGAATTGAATGTGACCAAGGCCACTCTTCTTACATTCAAATCTGGGTCTTCCA
ACGTTTTTAGAAAATCACCTATGCAGTTCTTGAGCAGTGGATCTTCTTGACATCTTGA
ATAAACTGACCTACTACAGCCGGTCCCTCTTTAGGGCATGCTCGAGTAAGGGCAGCT
ACACATTTGGCAATGGAATAGTAAGACTGCTTATGAGTAAGAGCTGTGCTCTGAGAG
TAAACTGGACCCGTTAGCATGCGCAGCAAATCCATGTATCCTAGATTGTTTGTTCCA
GTGACAACCAAAGCTTGGAAGAAGTCTAGCATGGCACTAAGAGCTCCTCCCTGCAG
CAGAGGTGACCTTACAAGTCCAATCAGTTCATTGAGAATAGATCCGCTTATCTTTGA
AAGGGAGGAGGGATATACTTTTGCCAGGGTAGTAAGGAAGCTGATAGCCATCTGGG
ACACGTGCATATCACTTTCGCTGATAAGAGGAGGGAGCTCATCCAGAACTGCATCAA
TCATGGCGGCCGTCAAACTGTCACTATAGTTTTTAATGAGAATATCTAGGGCAGAGA
GGGTCCCCAGTTTCAAAGCTCTCTGATTTTTCCTGAGAAATGAAGCAAGGATAGGGA
CTCCCTCTCCCAGCACAGGCCTCAGATCTATCTTCAAAGGTGACCCAGCAATCAGGG
TCAGTGCTTTCACTGTCGTTAGCCGGGTGATTTCATTCTTGAGTCTCTCCAAGAAAAT
CTGAAGTGTATTTGATAAGTCAGGGCCCAAATTGTCTCCAAGATTGCAAATAATCTG
TCCCATACAGGAAATAGCCCTCTCCTTGACTTCCTGATCAATGTCAGCTGCTTTTAAG
CGCTTAATTGTACAAGTGAAGAGATCTTTGATGTAAGGCGTTGCATCGAAGGAGGA
GGGTTGGTCCAGAGGACGGATTACTTTGACAAGCTGCTGAGTGACAAGAAGGGCTT
CTGATGTGATCTTGTAAAATGGGTCACCAACACAAGCCACCACTGGAGGGACCAAA
GCCTGAACATGCGGGTGGAAAACTTGCG-3' (SEQ ID NO:9). This clone is similar to a TATA-binding polypeptide (TIP120).

Another IEG nucleic acid clone was designated L048. The nucleic acid sequence of the L048 clone is as follows: 5'-TCGCCGCCCGAAGTCGCGCAGCTTCCCTGGCGAACG
CGGAAGCCCGAAGAGCGCCGTCCTCGGGCCCTGTCGGCGCTCAGGCCCCTTCGCGCG
CCTCCTCGCTCGGCCGGGACGTTGCTGTGGAGGCGTGAGGCGCCGGCGGTCGAGCA
CCTGGAGCGACGGTAGCCCGCGGCCTGCGGTTCTTCTCCTCCCCCGCCGCCCTCCCA
CCCGAGCTGCGGCGGGGCTCGGCCGCCTCGGTGCTTCTGCACGAACAAAGGAGGCC
CCCGCGGCGCCGGCGCAGCTCCATCTGCGGTCCGATCCACCCGGGCCCGCGGCGGCC
GCTAGCCAGCCCTTCCCGGAGGCCTCAGCCCGGCCCACCGCCCGGCGTCGCGCGCCA
GCTCGCTAGTGCATCCGGGCCCCGCAGGCACAAAAATATGGCTCAGGAGACTAACC
AGACCCCAGGGCCCATGCTGTGTAGTACTGGATGTGGCTTTTATGGGAATCCTAGGA
CAAATGGAATGTGTTCTGTTTGCTACAAAGAACATCTTCAGAGACAGCAGAATAGTG
GCAGAATGAGCCCAATGGGGACAGCTAGTGGTTCCAACAGTCCTACCTCAGACTCTG
CGTCTGTACAAAGAGCAGATGCTACTTTAAACAACTGTGAAGGTGCTGCTGGCAGCA
CATCTGAAAAATCAAGAAATGTGCCTGTGGCTGCCTTGCCTGTAACTCAACAAATGA
CAGAAATGAGCATTTCAAGAGAGGACAAAATAACCTCCCCGAAAACAGAGGTGTCA
GAGCCAGTTGTCACTCAGCCCAGTCCATCAGTTTCTCAGCCCAGTTCTTCTCAAAGTG
AAGAAAAAGCTCCTGAGTTGCCCAAACCAAAGAAGAACAGATGTTTTATGTGTAGA
AAGAAAGTTGGCCTTACAGGGTTTGACTGCCGATGTGGAAATTTGTTTTGTGGACTT
CACCGTTACTCTGACAAGCACAACTGTCCTTATGATTACAAAGCAGAAGCTGCAGCA
AAAATCAGAAAAGAAAATCCAGTTGTTGTGGCTGAAAAAATCCAGAGAATATAAAA
TTACTACATGTGAAGAGACTGAAACTTTGTTTTTATTTTAATATATCGTAGGAAAAC
ATTAAAGAGCAGATGCATGGCCATTTTCCTTTGATGTTCTCCAGAGTTTTGCTTTATA
CTTGTCTGTCATATAATTGATATTTTAGGATGTTTGGGTGTTTGTTACAGGCAGAATT
GGATAGATACAGCCCAACAAATGTATATGCCCTCCCCTCAGTAAAATTGGACAAAA
ATATGCACAGCAAATTGAAATACACATATACTAGGAACAAAATTTAGTTCCATGTGC
CAAACTGAATGAAATCTCTGCATGTTTGCAGCATATCTGCCTTTTGGGAATGTAATC
AAGGTATAATCTTTGGCTAGTGTTATGTGCCTGTACTTTAAAAAAATGGTACACCAG
AAAAGGACTGGCAGTCTACTACCATAGTCAAACTTCACCTTAATTTCGACATGCTTT

TGGAAGCAGGAAGAAAGCTACAAAACCAGTATTTGGTGCCATGTGTGAGCCTGGTT
AAATTGGTCTTCTAAAAGCTGTCAATTAGGACATTCTGCGAAAGGTAACATCACAAC
TGGTTCTGAGTAAAACCATCAAGTCAACAGCAGGGTGCCTGAGATAATCTTTGAAGC
TTATTGTGCTGGCCTGCACCAGAAGATATCTGCATTCTCATTACTAAAATTGTAGCAC
AGAACTGCACTAGGATTTGTTTACAAGAAGAAATTAAAACTCTACGTTTGGTTTTCA
CATATAGCAGCTCTGTTAAATAACATGCATCTGAATTTTAAGTTGCAAAGGTATCTG
AGCAGTTAGTTTTTCATGTGCATCTTTTGTTGAATGTTTTGGTTCAAGAAAGAATGTT
TAAAGCTTTTTAAAGACTTCAGTTCTTAATGTAACTGTACCCTTCTGCATGGAAAATC
ATAACCAACATGGCTGCAGTAGACTTCTTTAGTGGTATCCAGCACCACTTGCAGAGG
GCTGCTTTATCATATTGTATTTGGGTGTAGGACTCTAGTGTTCTTGGGTGTATTGCAT
GGGCTGCATTATCTACAGCATTGTACAATAACAACTAGAAAAGGCAGTATACTTCAC
TGATGCTTGTCTGGTAATATCACTTCTGTGTTATAATGGAAGGTTTTTTGTGATGTAT
GAAACTTGTGTTTTTTATATATAAATGAGTATAGTTAGATTAGTGTTGTGGTAATGCC
TGTTTTCATCTGTAAATAGTTAAGTATGTACACAAGGCACTACTTCTGATTTATTGCA
GTGTTCAGTCCTAGTTTTTCTTTATTAAAACATTCAGTTTTGCTTCAATTTTATGTACT
TTAGTTCTAAGTTAGATTTGCAGATGTGTACAGATAGTTCATATTTATGTATTGCACA
TAATCATGCTATTCAGCATTGATGCTATATTGTATTATGTAAATAATAAAAGCAGTG
TACAGAGGGAAAAAAAAACTCGTGC-3' (SEQ ID NO:10). In addition, the L048 clone contains an open reading frame (ORF) from basepair 414 through basepair 1055. This ORF encodes a polypeptide of 214 amino acid residues. The amino acid sequence of the L048 polypeptide is as follows: MAQETNQTPGPMLCSTGCGFYGNPRTNGMCSVCYKEHLQRQ QNSGRMSPMGTASGSNSPTSDSASVQRADATLNNCEGAAGSTSEKSRNVPVAALPVTQ QMTEMSISREDKITSPKTEVSEPVVTQPSPSVSQPSSSQSEEKAPELPKPKKNRCFMCRKK VGLTGFDCRCGNLFCGLHRY SDKHNCPYDYKAEAAAKIRKENPVVVAEKIQRI (SEQ ID NO:11). In addition, the L048 polypeptide was found to be cysteine rich, having a motif with distant homology to that of polypeptides with Zn^{++}-fingers.

Northern blot analysis using a sequence from the L048 clone revealed the presence of a 2.5 kb mRNA transcript. In addition, this analysis revealed that the expression of the L048 mRNA was strongly upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated L064. The following nucleic acid sequence is within the L064 clone: 5'-ATTCCAAAAATGCATAGATTACAAAGAAACACC AGACAAGCTCAAACTCAAGGATATTCTACAAATAAACCAGTACCTTCAAAATGCCAT GCTACCAGGTACAGACAGGCGAGANACTGTTCCACACTGAGGAAACTAACAAAGTA TCCATGAAGTCCATAATTGTGGGTCAAATCCAGGACCTGCAAAGGGGATTTGGGGAT AATTTTCAAAATTTGACTAAGGTCTGCAGAGTAGAGAGACGAGGTCAATGCCAATGT CCTGATTTTGACAGTAAGTATTTAAATATGCAGGAGAACAACCTAACCAAGAGGCTG CCAACACACTTCCTGGCTGTGGCACAACTAGATTTAAAACCAGCAATTTGTTGGTTC TTGTTCTCAAATATCAGTTACCTGCAAGCACTCCATCGTGAAAGGATTGAGAGCATG AGGTGATGTGTTGATGGTGAAAATGAGAACTGACTGAGCACAGGAAAGAGTGGCAT GATGGGCAGGGAAAGGGGAGACAAAGGTCACAAGAGCATGCAACACTCAGTGAAC TACAGGACACTCCAAAAGGCACTCTGCTGTCTAGCTTGGATCTGGAGGAGGATCAG NTATTAATAAGGGCCCTGGAAGGGNCAAAGCTAGCCTCCCAGCTGCTGGCTTCCCAT CTGCT-3' (SEQ ID NO:12).

Another IEG nucleic acid clone was designated L067. The following nucleic acid sequence is within the L067 clone: 5'-GCCACCACCATTGTTAATGGAGGGAGGCTCTCC CTTGTTATTTCTCAGAAGACTGAATGTCTGTACCAAAAGGCTCATGGCTTTCTCTGGG CCTTTCCATTTAAGGTTATAGTTTTTTATGTAGTGTTACTAAAATCTAGGCTTGTTACT AAAGTGGGCTTTGTAGTTATTGGTATCGGTGGATTTTTATGTTACTTGGAGTCCAGAA CAGGGAGAGCTCACCACAAACCTCTCCTTTCCCTGGACCAAACACCCTCTCTGTCCT GTGAACTCACCTTTTCTTCTCTGTGGGTCACTCCCATTACCACACTGGTGAGCGAGCC AAATGGATGAGAGACACAAAGACCGTAGTTCTTGAGAGACATTATTTTTTTCAACTT TGTTTTTTAAGAGATTTTATGTGTTGATTTGTTTTGGTTTGGTTTAAAGGGATTCATA GCTAACTTGGATTTTTGTTACCTCAGCTCTGGGAGAGGATTTTTGCTGAATGACTATT AATTACCTGAGCATTGTTGCTCTGAGGTCATGGCATGCTAGCCTATGTCTGTTACAGT

CTCAGGCTGCCCTTGTTTCCTCGTTCCTGTGCTATTGTGCTACACGCTCAAGGGGCCT
TGACTCTGCTTACACACATTAGGGGCAGTGTGAGTAAATGTGCAGTGTCCACACTTG
AGGACATGAATGTCTGCACTGTCACTTTGCTCTGGGTGTGAAGTCCCTGGTCCCCTTG
CTCCTGTAGCTTTCTTTTGATCGACTACTGGAACTCAACCCTGTGTACAAGAGCAGC
ACTGCCTCTGGTGGGTGGTGTTTGCAGCCAGGATTAGATGCCAGTCCTCGGGTTCCC
TGGCCTTGTTGGAAAGGTGTGCTTCCTTGAGGTCTGAGAATGGAAGGCTCTGCCTCA
CTCTAGCTAGGAGGCGCAATGGGAAAGTATGAGTTCAGGGCGTCAGGGCAGTGGCT
CCTGAAGAGCCAGCTGTGGACAGAGGGAGTGAGGCTTTATTTAAAGTGACAGGAAG
AAACATGGCGTTTTGGTATATTGGGAGCAATGCCAAGATTCCCTCCTGCCCTACATA
GGTCACAGACACCTTCCCAACCATCCCCTCCTCCACTTCCATAAATGAAGACAGCCC
TGATGACCCTCACCCCTTTTGCATAGGTCACTGGATCCCACTGTCCTTCCTCGGTGCT
TACACACTTTACAGACCCTTTAGGCGAGCCCTTGCATAGAGCGTTATCTCAGTGCTC
CATTCCAGTCCTGACTCCCTGTGGCCATTGAGACTTTGGATTTAAGAACTCACATTGC
TAGGGAGAGGGGCTTTGCTGGGAAAGGTGACTCCTCTGTAACCTAGCCTCTTGTGCT
CCTCCATGACAGAAATGCTGGGTGGAGTTTTACATTTGCCAATGGCCAGCTTGTGAA
TATCTTCATATACACTTTCTATTCATGTTACTGTAGTTTCTGTTTTGAAATAAAACTTC
TGAATGT-3' (SEQ ID NO:13). This clone is similar to a glucose transporter type III polypeptide.

Another IEG nucleic acid clone was designated L076. The following two nucleic acid sequences are within the L076 clone: 5'-CATATAAATGTACTTTATTGTTTTAAACAGAACG
AAAGAAGAGGCAGAAAACATTTGCATGTAAGTCCTAGCTTATAAATGTAGTTTTTAG
TGGTGGCATCTCTAACACGTCGTTCAGGGACTGTTTCCTTTTGCCTCCTTGTACTGTG
AGCACTGACACTTGAGAAAAGCACATCTGGCGGACATATGTCTCCAGAACTGGAAG
AACTTGGAGAGCAAACATTTTTCTTAATTCCTCTAAGTAATCTTTAGTAAAACAAAA
GATGATCTTTGGCATAGATTCATACTTTAAAGGCATTGATATGCATTTATATCAGGTA
AGCAACTATACAGATCTGCTGAGAGCTTTCAAAAGAATCTGTTATCAGCTGAAAGGA
AATAGGGGAAGCCTGAGTATTCAGGGTCAACTTAAGATTTGCAAGTTCAGTGTTGGG
GTCAACATACTAGATGTGGGAAGAACATCCAGGCAAGGTCTTAGTCCTGTATTCACC

TGGTTCTTGATTTCTGGAAGAAGCATCCATGCGCTAGGAAATGCTTATACAGCCGAG
GTAAATGCAAAAATGAGTAAAGTCACTTTTTCACTAACTTTGCCCAATAGGRAACAT
GCCTTTCTGATAAGTAGATACCATACTCTTTATTCTTGAATACTTTATATTGAGAGAA
GGTTGTAGTTGGTTAAAAGCAACTGGGAACTATAACTTCCTACTGATTTTTCCCTAGC
AGCACCAGAATTATATTCTGCAAATGCTATTCTCCCTTACATAGGAAATATCCTTCA
GACAAAATTGCCTTTCCATTCAGTCTCTTAAGAGYTTAATTTTGAATGGACTTTTCAA
AGTTACAAGCAAAGTCAAGTGTGGTGGTAGGAGCTAAGAGGCTGACACAAGTAGAT
GACTTGAATCCAGAAGTTCAAGACTAGCCTGGACAACATAGAGAGACCCAGTCTCA
AAATT-3' (SEQ ID NO:14) and 5'-GGCGGGGATCTCTCGGCTGGTAAGAAGGGG
CAGTGGTACCANGCGGGCACTTATTCAGTGTGCCAAGGATATCGCCAAGGCCTCTGA
TGAGGTGACGAGGTTGGCCAAGGAGGTTGCCAAGCAGTGCACAGATAANGCGGNTT
AGAACCAATCTCTTACAGGTCTGTGAGCGAATCCCAACTATAAGCACCCAGCTCAAA
ATCCTGTCCACAGTGAAGGCCACCATGCTGGGCCGGACCAACATCAGTGACGAGGA
GTCTGAGCAGGCCACAGAGATGCTGGTTCATAATGCCCAGAACCTCATGCAGTCTGT
GNAAGAGACTGTGCGAGAGGCCGAAGCTGCTTCAATCAAGATTCGANCAGACGCCG
GATTTACTCTGCGCTGGGTCAGAAAGACTCCCTGGTACCAGTAGGCACCTGGTCAGA
CCTGGCTGGTACACAGACCTCTGCTAATGANGANGTGACCATCTTGAGCTTCAGAAG
CCATTCAGAGTTGCCAAGGGGTGGNAAATCAATCCCTGGTTTCACACACCAAGAAA
GGGAATGGGGCCTCCTTCACATTAGAATAAACATTTATACTCTTGTCATGGGACACT
TTGAAAGTGTCTCTCCTACAAAACCCCTGGTACCTTTCAGGNTTACTCCNGGTNGCA
ANNTCCTCCCCCAAGGGGAATTTTTTACCAATAAAAGGCTCAAGGAATTAANGGCG
NTTGAAAACCAACNTNATCCAANGGGAAANGCCCCCNTGGCCTTCTGGCCCCCTTGG
GGGNACAATTTTTCNTCCCNCTGGGTGTTTTAAATGGGGTTTCAACCTTGGGGCTGG
NCCTTTTTCCNCCCCCCCTTTTAAGGGGCTTCCTCCGAAGGAACCTNAGAAAACTTN
AAGGGCCAAAGNTCCANTTTACNAATAACTGGG-3' (SEQ ID NO:15). This clone is similar to vinculin.

Another IEG nucleic acid clone was designated L082. The following two nucleic acid sequences are within the L082 clone: 5'-TTTTTTTTTTTTTTTTTTCCTCCCTTAAAAGAT

AAACTAATAAACTCTTCAATGGTCTTTTCAGTATAGTTCTTATGTAGTTTAACATAGC
TTATAAATTGAGTTTAACAATAAACTCAAGAAGATAATTTTATAAACCCTGTTTTCC
AATCTGTCATTTACTTAAATTATTTTGGTTGTTTTCCCTTTTTTTCCTTCTTTCTCACCC
CCTCCCTCTCCATGAAGATTCAGGTGCTTAACATATCATTTTTTTCCCTGCTGGAATT
TTAGCATTGATATGAACCATGGACAAGTATATTCTGCTGCCACAAAGACTGTAAAGT
GCTTCATTTCAACAGCTGAGGCAAGCCAAGTGATCATTAATAAAGCTTTTCTTGCTTC
CTTCAGTGGTGTTGGTAGTAAAATGGTAGGTAAAAGTTAGGCTGCAAGTTCAATAAA
TGAGATTTACCTATCATTCCACCCTTGTGTATTCATTCACCTATCCTGGTTCAAGCAG
TTTGAGTCAACTAGGCATTTAAAGGCATTGTGTTTATTACTTTATGGTTCCAACTTTA
CATACTTGTCAGGGATGAAGTCTGATAGGTTAAGGACAGTAGAAATTTCTGTGCAAC
AAGCAGCAAC-3' (SEQ ID NO:16) and 5'-TTTTTTTTTTTTTTTGGTTACAAAAGT
ATTTATTTTATAAAACTTGTATTTAAAATAGAGCTTATCTGTCTACTCACAAATCCTA
ATTTAAAACATAACACATTATCCTTAGCTAATCTGATGTTAACCTTTACAATCAACAC
TCATTTTTGTAATTTTATTAAGAACCTGTACTAAATGAAGTTTTTAATCAGAAAACAT
TCCCTTTTATCTTAAAAGTGCTTCTTAAATGAAGGCACCAACAAGAACTACTTTCAG
ATGGTACAGAATTTCTTATTTCTTGAAGACTCTGTGGTTGACCACTTCTTCATTAGTT
ACCTGCAGCAAGACACCTTCCTGCCAAAGGAAAAAAAAAAGTATCTGAAGAAGTTT
ATCATGTTTGTCCAAAGAACCTAAGTAACTTCAGTGGTGGTTTTAGGATTAAAGCAG
ACTCACTGATGTGTATACGCCCTGAATATCACATTTCTGGAAAGGCAGTAAAGCCTA
GAAATCAGAAGGCGGGCGGTTTTAAAGAAATTTCAATAGCCAACCTACAACANTTT
AGGGCAAAGATAATGGGCAAAAANTNC-3' (SEQ ID NO:17). This clone is potentially similar to a nRNP polypeptide A2/B1.

Another IEG nucleic acid clone was designated L094. The following two nucleic acid sequences are within the L094 clone: 5'-ACGATATMTAYWGARRTWYAWCTSTTHAC
TGAATMWHATGCACAAATATTAACTAGTRRTTTATTAAACAGATATSATTTAGAACA
AGACTTAAWKAAATACAAATCCTTAGGTACGRTTTAATATCATGTTCADGATGTTTG
AAGAGTTTAAAAAGAATCACTGATTAAGKKAAGCATCCBCACTTTTCTTTGAGAABC
CAAACCTTTTAGGNAAADACCCCATTCCAAATTTTGTCCCCHATTTCAGRCCKKCAG

AAAGTCTCTAACATSAAGAGTCCTCAACGGGGNGTAACTCAVAWCTCCTATCAAGT
GCAGTAACCTAGCTCTCCCGGDGGCCATGGCGT-3' (SEQ ID NO:18) and 5'-AAACT
AAACAGTGTTTTGTTAATTCTTCTGCATTCGGACTATTGCAGGCATTAGAGCATCCAG
AGCTACGAAGGGCTGGCTGCAGCAGCACCGCCCTTTGTAAGCCAGCAGACCAGCCT
TAACTGTGGGCTTGACTCCTGTGAGCTGGCCTCAGTGTGACTCAGAAATGTTTGATT
AGCAGATGAGAGAGCGAGGACACACCACGAGGGCTGCGTTCTCTTCCTCCAGCGCT
GTGCAGGACAGTTTCTTCTCACCCTAGCCTTTTTAAATGCACCAGAAGTACAGACAG
TTGCACTACACAAACCCTTTGAACACTTGTAGAAATCAGTCCACCGTAGATTAGACA
GAATCACCTTCCAATCCTTTGACTTCTTTTCCTTTCATTTGAACAATTGTATAATAATT
GATTATTGTCAAATTTTTGTCTGTGGTAGTATCGCTTTAATTTATCTTAGTACATCAA
CGTTTTGATTTAAAAAAGAATTAAAACAACAAAAAAAGTCACTTAGAAGCCATGAA
CTTTTTTTTTTNGATNGGGAAATTTTCTTGTTTNGAAAATTATCATTGGGGTTCCTCC
GGAAANCTTGTAAGATTGGNTTATAAGGTACCTGGGANGTTCANAACNGGTGGNTA
TACCCTTTTTTAAGGGAAATTAATGATTTNGAGTTTTTGGGCCAACTNCGGGANTGG
CAGGGAAACCANNCNGGGGNGGGGTTTAAATTNTGTGAGGGTTTTTTGGGCCTNAA
TTTTTTGCATAATTTTCACCTNGNAACCTTTNAANNCTNGGAAAAAAAAAAAAACNT
-3' (SEQ ID NO:19).

Northern blot analysis using a sequence from the L094 clone revealed that the expression of the L094 mRNA was upregulated in response to the multiple MECS treatment. Specifically, L094 mRNA expression was induced 7.3 fold by the multiple MECS treatment as determined from Northern blot data using total RNA from rat hippocampus (Table I). In addition, developmental studies revealed that the transcriptional expression level of L094 was upregulated between day E15 and E18, and downregulated at day 0. The expression then increases again during post natal development.

Another IEG nucleic acid clone was designated L097. The 5'-end of the clone obtained from the first library screen was used to design an antisense primer. Using PCR, L097 DNA was amplified and inserted into the pCR2.1 vector. The L097 clone is about 4.4 kb in length. Sequence analysis of the first 4060 bases from the 3'-end revealed the presence of a coding

region of at least 2351 bp. In addition, RT-PCR analysis revealed that the L097 clone was missing an adenosine at position 1166 from the 5'-end. The lack of this base results in a frame shift in the coding sequence. Further, the sequence at position 1358 was ambiguous. However, any base substitution at this particular position will not alter the encoded amino acid residue. Specifically, a serine residue will be encoded by the codon containing nucleic acid position 1358 regardless of the base at position 1358. The following nucleic acid sequence is within the L097 clone: 5'-TGCAGCCGCCCTTGGAACTGCATGTCAGGAAGCATCCCTTTGTGTA
TGTCTGTGCTATATGTCTCAAGAAATTTGTCAGCTCAATCAGGCTGCGCTCCCATATC
CGAGAGGTGCATGGGGCGGCCCAGGAGACCTTGGTTTTTACTAGCTCCATCAACCAG
AGTTTCTGCCTCCTGGAGCCTGGTGGGGATATCCAGCAGGAAGCCTTGGGAAACCAG
CTATCACTGACAGCTGAGGAATTTGTGTGTCCAGAAATTGATGTACGTAAGGGGGAG
GTTTGTCCTGGGGAAGCTCAGCCTGAGGTGGGGCTGAGGGAGTTGGAGGCCCCTGG
AGAAGCATGTGCCCCAGCCGTGCCCTTGGCCAACCCCCAGAGTGTCAGTGTTTCCCT
GTCCCCCTGCAAACTGGAAACCACTGTGGTCAATTCCGACCTCAACTCTCTTGGAGT
GGTTTCAGATGATTTTTTACTGAAAACTGATACCTCTTCTGCTGAGCCTCATGCTGCT
GCTGAGCTAACCTCAGACACACAGCATCGAGGCTCAGCCCAGACTCAGGGTGAAGA
AGTCACACTGCTGCTGGCCAAGGCCAAAAGTACTGGACCAGACTCAGAGAGTCCTC
CAAGTGGAGGGCAGAATGTGGGTGCTCTGCCAGCCAGTGAATCTGACTCTAACAGG
TGTCTCAGGGCAAACCCAGCAGAGACCTCAGACCTCCTTCCTACAGTGGCTGATGGA
GGAGACCTCGGTGTGTGCCAGCCTGACTCTTGCACGTCGTCCTCTGAGCACCACCCT
GGCAGCACAGCATTCATGAAGGTCCTAGACAGTCTCCAGAAGAAGCAGATGAACAC
CAGTCTTTGCGAGCGGATCCGGAAGGTTTATGGAGACCTGGAGTGTGAATACTGTGG
CAAACTTTTTTGGTACCAAGTGCATTTTGACATGCATGTCCGCACCCACACCCGGGA
ACATCTGTATTATTGCTCCCAGTGTCACTACTCTTCCATCACCAAAAACTGCCTTAAA
CGCCATGTAATTCAGAAACACAGTAACATCTTGCTGAAGTGTCCCACTGACGGCTGT
GACTACTCGACTCCAGATAAATATAAGCTACAGGCCCACCTTAAAGTTCACACAGAG
CTGGACAAAAGGAGTTATTCTTGTCCTGTATGTGAAAAATCTTTTTCAGAAGACCGA
TTGATAAAGTCACATATCAAGACTAATCATCCAGAGGTCTCCATGAATACCATTTCT

GAGGTTCTTGGGAGAAGAGTCCAGCTCAAAGGGCTAATTGGAAAGCGAGCCATGAA
GTGTCCGTATTGCGATTTCTATTTCATGAAGAATGGCTCAGACCTTCAGCGGCACAT
CTCNGCTCACGAGGGTGTGAAGCCCTTCAAATGTTCTTTGTGTGAGTATGCAACTCG
TAGCAAGAGCAACCTCAAAGCTCATATGAATCGTCACAGCACTGAGAAGACTCACC
TCTGTGACATGTGTGGCAAGAAATTCAAATCCAAAGGGACATTAAAGAGTCATAAG
CTCCTTCACACATCTGATGGGAAGCAATTCAAGTGCACGGTGTGTGACTACACAGCT
GCCCAGAAACCACAGCTGCTGCGACACATGGAGCAGGATGCCTCCTTCAAGCCTTTC
CGCTGCGCTCACTGTCATTATTCATGTAACATCTCTGGATCTCTGAAACGGCACTACA
ACAGGAAGCACCCCAACGAGGAGTATGCCAACGTGGGCAGCGGGGAGCTTGCAGCT
GAAGCCCTCATCCAACAAGGTGGTCTGAAGTGTCCTGTTTGCAGCTTTGTGTATGGA
ACCAAATGGGAGTTCAACAGACACTTGAAGAACAAGCATGGCTTGAAGCCAGCGAC
AGAGACTCCCGAGGAGCCCTCCACCCAGTATCTCTACATCACCGAGGCTGAAGATGT
TCAGGGGACACAAGCAGCTGTAGCTGCACTTCAGGACCTGCGATATACCTCCGAGA
GTGGTGATCGACTTGACCCCACAGCTGTGAATATCCTGCAGCAGATCATTGAACTGG
GTTCAGAGACTCACGATGCTGCTGCCGTGGCCTCCGTGGTTGCCATGGCGCCTGGGA
CAGTGACTGTTGTAAAGCAGGTCACCGATGAGGAACCCAATTCCAACCATACAGTC
ATGATCCAGGAGACTCTGCAGCAGGCCTCTGTGGAGTTGGCCGAGCAGCACCATCTG
GTGGTGTCCTCTGATGACGTGGAGGGCATTGAGACAGTGACAGTGTACACACAGGG
TGGGGAGGCCTCAGAGTTCATCGTGTACGTGCAAGAGGCTGTCCAGCCCATGGAGG
AGCAGGTCGGGGAGCAGCCAGCCACAGAACTCTAGAGAATCCCTGCCTCCTTTGGC
AGCCAGCCTTTGTGGGCCTGAAGACCTCCTAACCCACCAGGTCCATCCCTGGCTCTT
CTTGCCCACTGGCCCCAGATAAATTTCTCCATAACTGTCCTCTGTGTGGTCAAAGCCA
GGAGAGTATCATGAAGAGAGAGAGAGAGAGAGACTAGTCTCCGAGTTTTTTTTTT-3'
(SEQ ID NO:20). In addition, the following amino acid sequence is within the L097
polypeptide: QPPLELHVRKHPFVYVCAICLKKFVSSIRLRSHIREVHGAAQETLV
FTSSINQSFCLLEPGGDIQQEALGNQLSLTAEEFVCPEIDVRKGEVCPGEAQPEVGLRELE
APGEACAPAVPLANPQSVSVSLSPCKLETTVVNSDLNSLGVVSDDFLLKTDTSSAEPHAA
AELTSDTQHRGSAQTQGEEVTLLLAKAKSTGPDSESPPSGGQNVGALPASESDSNRCLR

ANPAETSDLLPTVADGGDLGVCQPDSCTSSSEHHPGSTAFMKVLDSLQKKQMNTSLCER
IRKVYGDLECEYCGKLFWYQVHFDMHVRTHTREHLYYCSQCHYSSITKNCLKRHVIQK
HSNILLKCPTDGCDYSTPDKYKLQAHLKVHTELDKRSYSCPVCEKSFSEDRLIKSHIKTN
HPEVSMNTISEVLGRRVQLKGLIGKRAMKCPYCDFYFMKNGSDLQRHISAHEGVKPFKC
SLCEYATRSKSNLKAHMNRHSTEKTHLCDMCGKKFKSKGTLKSHKLLHTSDGKQFKCT
VCDYTAAQKPQLLRHMEQDASFKPFRCAHCHYSCNISGSLKRHYNRKHPNEEYANVGS
GELAAEALIQQGGLKCPVCSFVYGTKWEFNRHLKNKHGLKPATETPEEPSTQYLYITEA
EDVQGTQAAVAALQDLRYTSESGDRLDPTAVNILQQIIELGSETHDAAAVASVVAMAPG
TVTVVKQVTDEEPNSNHTVMIQETLQQASVELAEQHHLVVSSDDVEGIETVTVYTQGGE
ASEFIVYVQEAVQPMEEQVGEQPAT EL (SEQ ID NO:21). Using tblast2x algorithms, nine Zn^{++}-fingers were identified by homology to motifs of Zn^{++}-finger containing polypeptides (accession # PIR2:A32368, S03677, A29634, S06571, and A60392). The presence of the multiple Zn^{++}-finger domains suggests that the L097 clone is a transcription factor, however, the size of the encoded polypeptide is in excess of 700 amino acids.

Northern blot analysis using a sequence from the L097 clone indicated that the L097 mRNA transcript is rather rare. In addition, this analysis revealed that the expression of the L097 mRNA was very weakly upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated L099. The following four nucleic acid sequences are within the L099 clone: 5'-TGGATCTACTTGTTAATGGTTTCATGGAAGC
AATCAGCAATATGTGATATGAACTGCTGCATTACTTATTATACTCGTGGAACTGAGA
TATTTARMSRSMGCTTWWYTTTTTTTTTTYTTAGTGTAAAATACTTAAGCGTTTCCAC
TATTGGAAGAAAAGCATATATGGGTATTTTGTATTGTAACTTGTTTAAAAGGACAGT
CTTTTTTAAYCTTCCCACTTAAATGCTTTTAAAATATGTAATACAATTTGAAGCTTGT
TTAAAAATAGAATTAAATGTCTTAWATAGKGCTACKGTTTTGGAATTAGAAAGTGAT
CAAATACAAAACATTTTAAAATTAAGCCCAGAAAACAAAATAGTGTTTAAAGTTAG
TTTAGTATAAAAGAAATTTATAAGATTTTTTCTTCAATATAAGATACCTCACTTGAAA
ATAAAGAAAGCACAGCACATTAAAGTAATTCTCATGAGAACACCCCATTAGAATAA
TTGCTAAATCTAGGACACCTTTTGAGTTGTGAGTTTGTGATACATGTAGTCACCATTA

GCTTTTCTGCTGGAAGGACTTCCCGTAGTAATTTTAAGGCAGTGTAATAGTTCAATTA
CCCCACAGTTTCTAACCTGGGAAGGCAGTATGTGAATGGTCCCTTCTGCAACTACGG
AAACACATTAGCTACATTGAGCATAACTCGATTGATAATTTTGCCAGTGCATATAGT
TTTATGATTAAAATTGCTGTGGTTGGTTGCATTACACGACACACAAAACTGTCCTCTA
CCTCACATGAAATAAATATTTTATATGGTTTTACTAAAAAAATGACTCATCTATCTGG
TTACTTAGTTTACAAATTTTGGATTATATTTATTGAAACATGACATACTGTGCTCTTA
GCTTATACCTCAATCGTATTTTGTGCTGTTTGCCATTTTCATGCCTTGTATATAACTTG
TATAGATTGGATGATATTCCCAATAAACACTTTTAATKCCAAWRAAAAAAAAAAAAA
AAAAA-3' (SEQ ID NO:22); 5'-TAATGTTTATGATACAAAGCTACTCACTCTG
GAGCCTTCTCATTACAGAATCTCTTGACTTTTATACACCCAGCCTGTTGTTACTTTGT
TCAGGTTGCAGAATGAGTTTCCTCTGGTTTCCTCCTAGAGGAGTTTTCCTGATGAAAT
GCTAGTAGCACCTCCCCGACATACAGCGGGTGGGTGGGGCACACTTTGCTGTGCTCT
GATGGTACACACAAGAAGCAGTTGTAATTTGTCTTTCTGTTTAAGAGTGACCATAGC
TAGATATGTGTGTGTGACTTCAGAAAATTAAAATGCTTTCCGAACTTTTCCTGTTAAT
AGAGGTGTGAAGTACTCATTCATGTGCATGAGGAAAGTGGATTCCACGGACGCACA
CCGCTTCCTATGTAACTCACAATGCTCTGTACAGTTTTTATATGTAGTCTTACAAAGG
TCTTATGAAATTTATATAATGGATTTTTTCTTTTAAATTATAAAATACTAAATATCTT
AAAGATTGTTTTGGACTTTTGTATGTTTAAATGTTATCTTAAAACTTGCACAAATGGA
CCATGATGACTCTTTGATCTTAAAATCAGGAATTTACAGTCAGCTAAGAAAAATGTG
GATAGGTTAATAATCCACAGTGGGAGTATCTGCTAGGAGCAGGAATTGTAGATGAC
ATGAATTCCGTGATTTGAGGAAGGGCAGCCTCTGCACTTTTCTTTGTTTTTGTTTTTT
GCACATGAAGTCTGACATTTTTACCATCGAATTTCACATTACTAGATGGTTGGCTTGG
GATTTACCTAGGGGAAATTCTTAGCAACTTTGTACTTTGTTGTTTTTGTTCTGTTTGGT
CTCCAGCTTGCAGAGACCCTCTTGCCTCTGTCTCCCAAGTGTTGGGTTGGCAGGATG
AGCCCCACCACCGCTGGCCCTGTGCAGTTCTTTTGGGATGTCCCTGAAAGCAGCTGT
GGCATTATCTTCTGTTTCATGTGTCCCGAGCTGTCTCATGGTACTACATGCAGTGACC
TGAGATCTGCGTTAAGGAATAACTTAGGAGAAAACGGCTGTCACTGTCCTCCCCGCT
GTGAGACACCAGAGTTATCACACCTGTTATGGTCATACTTTGTGTTATGATACTGAT

GTCTAAGGCAATTTTTCTACTTTCCAAAAGGGAGTTTGTTTCCTAAATATATTGTGAC
CTAAATGTGGTTTTATTCTGCTATGTTCTATAATTTATGTATTGACTTTTGTAACCTCC
TTGGGAGAAACATGTTAAGTGGCACAGGGACCATATATGTCATTTTATTTAGCTCTG
GAGAAGGAAACCACAGGCGTTTGTAAAATAGCATTAGCTTAGATGTCAGTTCATTGT
GCTTGGCTGTGTGGGAGGCAGACTCAAGGACTTGCACCATTTATTTTTCTGACAGAA
GTGTTCTGCTTATGTGCTGCTTAGTAAGTGTGATTTTTCTAGTCTTGATGAAACTTGC
CTCGTGACATTGTTGAGCGTAGTCTTCACTTTCCAGAAGATGAAATGATGTGCCATC
ATTTTCTGTCTAAACTTCCTTTAAAGTAATTTTTAATCAGCTGTAAATATCATATCTC
CTACTGTTGAAAGTAACTTTAATTTACATTGCACCATATAGCTTGAAAACCAACTTTG
AAATTCTGTACTCCTCCACAAGTGACCTCCGCTAAAATACCCATAGGAAGCTTACTT
TGTGCATGCNTGCTTTGTGTGCCGGTTGCCGTCCTAANGGTTGCTTTGGG-3' (SEQ ID
NO:23); 5'-TTTTTTTTTTTTTTTTTTAGTGTAAAATACTTAAGCGTTTCCACTA
TTGGAAGAAAAGCATATATGGGTATTTTGTATTGTAACTTGTTTAAAAGGACAGTCT
TTTTTAATCTTCCCACTTAAATGCTTTTAAAATATGTAATACAATTTGAAGCTTGTTT
AAAAATAGAATTAAATGTCTTATATAGTGCTACTGTTTTGGAATTAGAAAGTGATCA
AATACAAAACATTTTAAAATTAAGCCCAGAAAACAAAATAGTGTTTAAAGTTAGTTT
AGTATAAAAGAAATTTATGAGATTTTTTCTTCAATATAAGATACCTCACTTGAAAAT
AAAGAAAGCACAGCACATTAAAGTAATTCTCATGAGAACACCCCATTAGAATAATT
GCTAAATCTAGGACACCTTTTGAGTTGTGAAGTTTGTGATACATGTAGTCACCATTA
GCTTTTCTGCTGGAAGGACTTCCCGTAGTAATTTTAAAGNAGTGTAATAAGTTCAAT
TANCCCACAAGTTTCTAANCTGGGAAAGNAANTATGGTGAATGGNCCCTTCTGCAAC
TACGGGAACACA-3' (SEQ ID NO:24); and 5'-TTTTTTTTTTTTTTTTTTTTGGCATTAA
AAGTGTTTATTGGGAATATCATCCAATCTATACAAGTTATATACAAGGCATGAAAAT
GGCAAACAGCACAAAATACGATTGAGGTATAAGCTAAGAGCACAGTATGTCATGTT
TCAATAAATATAATCCAAAATTTGTAAACTAAGTAACCAGATAGATGAGTCATTTTT
TTAGTAAAACCATATAAAATATTTATTTCATGTGAGGTAGAGGACAGTTTTGTGTGT
CGTGTAATGCAACCAACCACAGCAATTTTAATCATAAAACTATATGCACTGGCAAAA
TTATCAATCGAGTTATGCTCAATGTAGCTAATGTGTTTCCGTAGTTGCAGAAGGGAC

CATTCACATACTGCCTTCCCAGGTTAGAAACTGTGGGGTAATTGAACTATTACACTG
CCTTAAAATTACTACGGGAAGTCCTTCCAGCAGAAAAGCTAATGGTGACTACATGTA
TCACAAACTCACAACTCAAAAGGTGTCCTAGATTTAGCAATTATTCTAATGGGGTGT
TCTCATGAGAATTACTTTAATGTGCTGTGCTTTCTTTATTTCAAGTGAGGTATCTTAT
ATTGAAGAAAAAATCCATAA-3' (SEQ ID NO:25). This clone is similar to sno I.

Another IEG nucleic acid clone was designated L100. The L100 clone is 2924 bp in length and has a nucleic acid sequence as follows: 5'-TGCGGCCGCCGGGGCCGGG
GCTGAGCCAGTCTCTCCCGCCGCCGCCGGACGCGCAGACCTGGGCAGGCTGCACCG
ACGGCCGCCTGGCCGAGCGCACTGCAGGTCGCTGCGCGCGCTGCGACCCCGGGGCC
CGGACGCGAGTGGCTGCGGTGTCCTGGGCGAGCACTGCTAGTTTAGGCCGTCTGTCC
TCAGCTGCTTGGAACCCCTACATCCCACCATGGCTGGGATACAGAAGAGGAAGTTTG
ACCAGCTGGAAGAGGACGACTGCAGCTCCTCCTCCTTGTCCTCTGGCGATCTCTCTC
CCTCTCCTCCCAGCTCTTCTGCCTCCCCTGCCTGGACCTCTGAGGAGGAGGGACTGG
GTGATCAGCCACCCCAGCCTGATCAGGACTCCAGTGGCATCCAGAGTTTAACGCCCC
CATCCATCCTGAAGCGGGCTCCTCGGGAGCGTCCGGGTCACGTGGCCTTCGATGGCA
TCACTGTCTACTATTTCCCGCGGTGCCAGGGATTCACCAGTGTGCCCAGCCATGGTG
GCTGTACCCTGGGCATGGCTTCTCGTCATAGCACCTGCCGCCTCTTCTCCTTAGCCGA
GTTTAAACAGGAGCAGTTCCGGGCTCGGCGTGAGAAGCTCCGTCGGCGTTTAAAGG
AGGAGAAGCTAGAGATGCTGAAATGGAAGCTTTCAGTGTCCGGAGTTCCGGAGGCA
GGGGCAGACGTGCCGCTCACAGTGGACGCCATCGATGACGCTTCTGTAGAGGAGGA
CTTGGCAGTGGCCGTGGCAGGTGGCCGCCTGGAGGAAGCGAATTTCCTACAGCCCTA
TCCACCTCGGCAGCGACGGGCCCTACTTCGCGCTTCCGGTGTTCGAAGGATTGACCG
AGAGGAGAAGCACGAGCTGCAGGCGCTACGCCAATCCCGGGAGGATTGTGGTTGTC
ACTGTGATGGCGTCTGTGACCCTGAGACCTGCAGTTGCATCCTGGCGGGCATTAAAT
GCCAGATGGATCACACGTCCTTCCCCTGTGGCTGCTGCAGCGAGGGCTGTGAGAACC
CCCATGGTCGAGTGGAATTCAATCAGGCGAGAGTTCAGACACACTTCATCCACACGC
TCACCCGCCTGCAGATGGAGCAGGGTGCGGAGAGTTTGGGGGACCCGGAGTCCCCC
ATGGAGGACGTTCCTGTCGAACAAACCGTGGTTTCCCCCTTTCCTCCTTCCAAACCCA

CTATGAGCAATGACCTGGGGGACAGCAGCTGTGGCAGCGACATGACAGACTCTTCC
ACGACCTACTCCTCTGGCGGCAGTGGCAGCCGCAGCGAGGCTCCGAACCATCTTGCC
CACCCCAGCCTGCCAGGTTCCAGCTTCCGGTCTGGCATAGATGAAGACAGCCTGGAA
CAGATCCTGAATTTCAGTGACTCTGACCTCGGTATTGAGGAAGAAGAGGAGGAGGG
AGGGAGTGTGGGCAACTTGGATAACCTCAGCTGTTTTCATTTGGCTGACATCTTTGG
TACCGGTGACCCCGGCAGCCTGGCTAGCTGGACACACAGCCAGTTTGGCTCTAGCCT
TCCATCGGGCATCCTAGATGAGAATGCCAACCTGGACGCCAGCTGCTTCCTAAGCAG
CGGACTCGAAGGGTTGAGAGAAGGTAGCCTCCCCAGCAGTTCTGGGTCCCCTGAGG
GGGAAGCCGCCCAGAGCAGCTCCTTGGACCTCAGTTTATCCTCCTGTGACTCCTTTG
AGCTTCTCCAATCTCTGCCAGATTATAGTCTGGGGCCTCACTATACTTCCCGAAGGGT
ATCTGGCAGCCTGGACAGCCTTGAGACCTTCCACCCTTCGCCCAGCTTCTCTCCACCG
AGGGATGCCAGCTTCCTGGATTCTCTCATAGGCCTGTCTGAGCCGGTTACAGATGTC
CTGGCGCCCCTTCTGGAGAGCCAGTTTGAGGACACTGCTGTGGTGCCTTTGGACCCT
GTGCCTGTGTAAGGATTGAGATGACTTTTTCCTGCCCTGAGACCCTGTTGCTGCTTTT
TATGTGATCTTGGTGTCCCCCAAGGTCTGTGTATGTAACGGTCTCCCGTGGGCTGGTT
CTGCCCCCGTGCCATGTGGGCAATCCTCTATTTTTACAGTAACACTCTAGATTTATTT
ATTTTTTTATGTTTTTCTGTACTGAAGGGAGGGTGGGAAGGGTATCCCTCTTTCAATG
CCTGGCCTCTATGTCCAAACAGAGGTCTCCCACCTCCTACTGTATGCCTGGAGGAGG
AAGGGGCGGGGTTCACATCCCCTCTTTCTGTACTGTAAAATGCTCCTTGGTCCAAAG
ACAGCTGAAAAGCAGGCCTTAGGGTTTCCTGTGGACCGTGGGAGCTAGGTCTTCTGG
ACTCTGAAGATGTAATTTATTTCTGTAATTTATTTGGGGACTGAGACAGCAGTGGTT
GGGCCTCTCTGGCAGGTGGGCGGTGTTGAGGCAAAGTCTTCGGTGTCCCCCGCCGGT
CTGGGCTTCGGTGTGGCGTGTAGGTTCGAGCTGAGCAGACGGAGGCTGTGCTTGACC
ATCGGTGATCAAAACTCCCTCTGCCCCCTGCCCAGACGCTCTAACATGCCCTCTGTCC
ATTTCCCTCTCCCCAAGGCCATGGGTTATAAAGGCCCTATGTAGGATGGGGAGCCAG
AGGCCCTAAGACATGAAGCACACCCCAGATCACTGTCTCTAGCCTTTCTGGGCACTG
AATCCATCCTGACCCACCACACACCCCCCGGCCAGTTGGCAAGAAAGAGGTGGCTCT
TGGGGGCTTTTATGCCCTTCATTAGCTGATGTTGGATTTTATATGCATTTTTATATTGT

CTCTAAGTGTCAGAACTATAATTTATTCATTTCTCTGTGTGTGTGTGCCAAGAAAC
GCAGGCTCTGGGCCTGCCTCCTTGCCCAGGAGGCCTTGCCAGCCTGTGTGCTTGTGA
GAACACATTGTACCTGAGCTGACAGGTACCAATAAAGACACTCTATTTTTAAAAAAA
AAAAAAAAAAAA-3' (SEQ ID NO:26). In addition, the L100 clone contains an ORF from basepair 145 through basepair 1890. This ORF encodes a polypeptide of 582 amino acid residues. The translational start site was assigned to the first methionine residue in the ORF. The amino acid sequence of the L100 polypeptide is as follows: MAGIQKRKFDQLEEDDC
SSSSLSSGDLSPSPPSSSASPAWTSEEEGLGDQPPQPDQDSSGIQSLTPPSILKRAPRERPGH
VAFDGITVYYFPRCQGFTSVPSHGGCTLGMASRHSTCRLFSLAEFKQEQFRARREKLRRR
LKEEKLEMLKWKLSVSGVPEAGADVPLTVDAIDDASVEEDLAVAVAGGRLEEANFLQP
YPPRQRRALLRASGVRRIDREEKHELQALRQSREDCGCHCDGVCDPETCSCILAGIKCQ
MDHTSFPCGCCSEGCENPHGRVEFNQARVQTHFIHTLTRLQMEQGAESLGDPESPMEDV
PVEQTVVSPFPPSKPTMSNDLGDSSCGSDMTDSSTTYSSGGSGSRSEAPNHLAHPSLPGSS
FRSGIDEDSLEQILNFSDSDLGIEEEEEEGGSVGNLDNLSCFHLADIFGTGDPGSLASWTH
SQFGSSLPSGILDENANLDASCFLSSGLEGLREGSLPSSSGSPEGEAAQSSSLDLSLSSCDS
FELLQSLPDYSLGPHYTSRRVSGSLDSLETFHPSPSFSPPRDASFLDSLIGLSEPVTDVLAPL
LESQFEDTAVVPLDPVPV (SEQ ID NO:27). This amino acid sequence was found to contain numerous cysteine residues, forming a motif that has features of a methalothionein-like motif. Alignment analysis revealed that the L100 methalothionein-like motif exhibits higher similarity with the methalothionein motif from *C. elegans* than with the methalothionein motif from mouse.

Northern blot and *in situ* analysis using a sequence from the L100 clone revealed that L100 mRNA is weakly expressed in wild-type rat brain. For *in situ* hybridization, Dig-labeled cRNA probes were used as described elsewhere (Kuner et al., *Science* 283:5398 (1999)). Specifically, this weak L100 mRNA expression was observed in the pyramidal cell layers as well as the dentate gyrus of the hippocampus, thalamus, cortex, cerebellar granule cell layers, and several fiber tracts including the fimbria hippocampus and the cingulum. In addition, Northern blot analysis revealed that the expression of the L100 mRNA was strongly upregulated in

response to the multiple MECS treatment. Specifically, L100 mRNA expression was induced 17.2 fold by the multiple MECS treatment as determined from Northern blot data using total RNA from rat hippocampus (Table I).

The mRNA expression pattern of L100 demonstrated a compelling overlap with neuronal populations known to release Zinc into the synapse via synaptic vesicles and to take-up Zinc post-synaptically. Briefly, synaptic release and uptake of Zinc may participate in the induction and maintenance of epileptic seizures and the neuronal cell death following epileptic seizures and ischemia. The L100 metallothionine-like motif most likely enables the L100 polypeptide to bind Zinc or other divalent cations *in vivo*. The expression of L100 mRNA in Zinc-containing neuronal populations in the brain indicates that L100 polypeptide may sequester Zinc in brain.

In addition, when acute seizures were induced by kainate treatment, the expression of L100 mRNA was strongly upregulated (Tables II and III). Kainate-induced seizures is a model used to study epilepsy. Briefly, 300-350 g male Sprague-Dawley rats were intrapertoneally injected with either 10 mg/kg body weight of kainate or PBS. RNA samples from the hippocampus, cortex, and cerebellum were prepared from treated rats at 1.5, 6, and 24 hours post-injection. This RNA then was used to measure mRNA expression by Northern blot and RT-PCR analysis. Control mRNA measurements included c-fos, GAPDH, NO-38, and ATF-4 for the Northern blot analysis, and Hsp70, c-jun, Zif268, c-fos, Clathrin, and β-actin for the RT-PCR analysis. A Phosphoimager FLA2000 (Fuji) was used to analyze the data, which was expressed as the Integral PSL - background PSL (1D evaluation with Aida version 2.0).

At six hours following kainate injection, strong upregulation of the L100 mRNA was observed, by *in situ* hybridization, in the dentate gyrus and areas CA3 and CA4 of the hippocampus as well as the associated entorrhinal cortex, the cingulum, and fimbria, which are brain areas known to be highly excited in and which mediate Kainate-induced seizures. Moderate upregulation of the L100 mRNA also was found in the thalamic nuclei, temporal, parietal, frontal, medial orbital, and cingulate cortex as well as in the cerebellar granule cells. Thus, the data presented herein indicates that L100 participates in cellular mechanisms mediating kainate-induced epileptic seizures and the consequent neurodegeneration.

Table II. mRNA expression normalized to GADPH expression

Clone	1.5 hour PBS	1.5 hour kainate	6 hour PBS	6 hour kainate	24 hour PBS	24 hour kainate
Hippocampus:						
L100	4622	85251	7847	15444	3940	16551
L119	2816	69982	4597	11519	2787	12944
Cortex:						
L100	-	-	81	290	86	131
L119	-	-	255	1262	538	505

Table III. Fold increase in mRNA expression upon kainate treatment

	Hippocampus			Cortex		
Clone	1.5 hour	6 hour	24 hour	1.5 hour	6 hour	24 hour
A013	9.8	-	-			
L094	3.6	-	-			
L100	18.44	1.97	4.20		3.58	1.52
L119	24.85	2.51	4.64	-		
R113	2.0	-	-			
R286	-	-	-			

In addition, when acute seizures were induced by pentylenetetrazole (PTZ) treatment, the expression of L100 mRNA was strongly upregulated (Tables IV and V). PTZ-induced seizures is a model used to study epilepsy and ischemia. Briefly, 300-350 g male Sprague-Dawley rats were intrapertoneally injected with either 50 mg/kg body weight of PTZ or PBS. Total RNA samples from the hippocampus, cortex, and cerebellum were prepared from treated rats at 20 minutes, 6 hours, and 24 hours post-injection. This RNA then was used to measure mRNA expression by Northern blot analysis. Control mRNA measurements included c-fos and

GAPDH. A Phosphoimager FLA2000 (Fuji) was used to analyze the data, which was expressed as the Integral PSL - background PSL (1D evaluation with Aida version 2.0).

Table IV. mRNA expression normalized to GADPH expression

Clone	20 min PBS	20 min PTZ	6 hour PBS	6 hour PTZ	24 hour PBS	24 hour PTZ
Hippocampus:						
L100	534	1637	854	1992	966	1903
L119	342	965	-	-	-	-
Cortex:						
L100	958	2719	1162	3740	1175	1825
L119	577	1605	-	-	-	-

Table V. Fold increase in mRNA expression upon PTZ treatment

	Hippocampus			Cortex		
Clone	20 min	6 hour	24 hour	20 min	6 hour	24 hour
L100	3.1	2.33	1.97	2.84	3.22	1.55
L119	2.82	-	-	2.78	-	-
R113	-	2.0	-			
R286	-	2.6	-			

In another study, the expression pattern of L100 and L119 was determined using two models for ischemia. Briefly, neurons degenerate in brain and spinal cord after acute insults (e.g., stroke, cardiac arrest, and trauma) and during progressive, adult-onset diseases (e.g., amyotrophic lateral sclerosis, and Alzheimer's disease). Impaired energy metabolism plays an important role in neuronal cell death after brain ischemia, and apoptosis has been implicated in cell death induced by metabolic impairment. The irreversible inhibitor of succinate

dehydrogenase in the mitochondria, 3-nitroproplonic acid (3-NP), inhibits oxidative phosphorylation and causes intracellular hypoxia. Thus, one model used to study ischemia involves intrastriatal injections of 3-NP, which is known to produce selective cell death similar to that observed in transient ischemia and Huntington's disease (McLaughlin *et al.*, *J. Neurochem* 70:2406-2415 (1998)). The other model is a global ischemic paradigm that involves a 15 minute insult by complete occlusion of the carotis.

In the 3-NP study, 220-300 g Wistar rats were intraperitoneally injected with 20 mg/kg body weight. Three hours post-injections, the brain was removed and total RNA prepared. In the global ischemia study, 220-300 g Wistar rats were received a 15 minute insult (bilateral occlusion of the Carotis/arterial pressure = 35 mm Hg). One hour later, the rats received a reperfusion followed by immediate brain dissection and total RNA preparation. Untreated rats were used as controls for each study. Ten (10) μg of total rat brain RNA (without cerebellum) was loaded per lane and blotted. Probes were prepared from the 3' untranslated regions of L100 and L119. The Northern blot data was collected using a Phosphoimager (FLA2000 Fuji, Tina software) and expressed as PSL - background.

L119 mRNA expression was upregulated 6-fold by global ischemia while L100 mRNA expression was not inducible by global ischemia (Table VI). This result indicates that only seizure related stimuli alter the expression level of L100 and that L100 is not a general marker for stress response of the cell like c-fos.

Table VI. mRNA expression after 3-NP or global ischemia treatment.

Probe	Untreated	3-NP	Global Ischemia
c-fos	18.1	26.64	216.22
GAPDH	487.02	587.51	593.31
L100	30.95	43.82	40.15
L119	55.48	41.94	332.73

Northern blot analysis using multiple tissues from rat revealed that the expression of L100 and L119 mRNA was not brain specific (Table VII). Briefly, fragments from the 3' untranslated region of L100 and other IEG clones were labeled with ^{32}P-dCTP. The denatured probe was hybridized with 10 μg total RNA from rat brain, liver, lung, muscle, intestine, eye, heart, testis, and kidney in the Quik Hyb-solution (Stratagene) at 68°C and washed with 0.1X SSC at 60°C. For L100, after one day of exposure, signals were detected at the 3 kb position in brain. In addition, a weaker signal was detected in heart and a faint signal detected in kidney. A strong signal was detected in testis but this signal was at a position corresponding to a size smaller than 3 kb. For L119, a strong signal was detected in heart and weaker signal in brain. In addition, only very faint signals were detected in liver, kidney, and testis.

Table VII. mRNA expression in various rat tissues.

Probe	Brain	Liver	Lung	Heart	Kidney	Muscle	Intestine	Testis	Eye
A013	(+)		(+)		(+)		(+)		
L094	+		+	(+)	+	(+)	+		
L100	+++			++	+			+++(*)	
L119	++			+++					
R113	(+)	(+)	(+)	(+)	(+)	(+)	(+)		
R286	+++	(+)	+++	(+)	+	(+)	(+)		++

(*) smaller transcript

Another IEG nucleic acid clone was designated L111. The first round of screening produced a clone (designated L111-5) that contained a 3.0 kb fragment of L111. A second round of screening using the coding region of L111-5 as a probe produced several additional clones. The following nucleic acid sequence is within the L111 clone: 5'-ATTCGGCACGAGCCAGAG

TGAAGGGGCATGGAGAAGTGGACGGCCTGGGAGCCGCAGGGCGCCGATGCGCTGCG

GCGCTTTCAAGGGTTGCTGCTGGACCGCCGCGGCCGGCTGCACTGCCAAGTGTTGCG

CCTGCGCGAAGTGGCCCGGAGGCTCGAGCGTCTACGGAGGCGCTCCTTGGCAGCCA

ACGTAGCTGGCAGCTCTCTGAGCGCTGCTGGCGCCCTAGCAGCCATCGTGGGGTTAT

CACTCAGCCCGGTCACCCTGGGAGCCTCGCTCGTGGCGTCCGCCGTGGGCTTAGGGG

TGGCCACCGCCGGAGGGGCAGTCACCATCACGTCCGACCTCTCTCTGATCTTCTGCA

ATTCCCGGGAGGTACGGAGGGTGCAAGAGATCGCCGCCACCTGCCAGGACCAGATG

CGCGAACTCCTGAGCTGCCTTGAGTTCTTCTGTCAGTGGCAGGGGCGCGGGGACCGC

CAGCTGCTGCAGAGCGGGAGGGACGCCTCCATGGCTCTTTACAACTCTGTCTACTTC

ATCGTCTTCTTCGGCTCGCGTGGCTTCCTCATCCCCAGGCGTGCGGAGGGGGCCACC

AAAGTCAGCCAGGCCGTGCTGAAGGCCAAGATTCAGAAACTGTCTGAGAGCCTGGA

GTCCTGCACTGGTGCCCTGGATGAACTTAGTGAGCAGCTGGAATCCCGGGTCCAGCT

CTGTACCAAGGCCGGCCGTGGTCACAACCTCAGGAACTCCCCTGATCTGGATGCAGC

GTTGTTTTTCTAAGAGCATCCTCTAGCTGTGTGGAATGTTCTAGATTCGCAGCATCCA

CAAGGAAGTGCTACATGGGCGGAGTGCAAAGGATTTCAGAAGCTCTTCTTGCAGGG

CATCAGTCCGTAGCTCCTTGTGTGTGCGAAAGACTTTTCACTTGTGTAATCCCAACTG

AGTATGTGACCCTAAACAGTCACTTTGGGGACTCCCCAAATCCTTTTTAGCTGCACA

CAGCTTGTCAGACTGTCCTTCAATTAGAGTTATTGGGGTGGGGGGGCTTGATGGCTT

GAGTAATAGAGGTCTGGCGAGGTGTCTCCCTCTTGGACCTCTTATGTGTTGTTACTAG

AATCCTGAGATTCTCAAATGTTGGTGAGAGGAGACTTTTACTTTTCAACTTTGCTTCG

GCAGTTTCCGATACACAGGACTCCAGAATCCAGAACAAGAAAGAAGAACCTTGTGT

TTGTAGGGTGTGCAGACCCAGACGGGGCCGAGGAGCTGACTTGCTCAGCTCTCACAC

GCAGCCAGTTTATCCACTCACAGACCAAACCTGGCTACTGCATAGACTGTTCCAGTG

TGGCTTCAAATCCACACCTCTAGGTACCCTGAGAAGGAAAGCCACCTGAAGAGTCA

CTCTAATCCCAACACGCTCACCCCCTTCACGTCCATAAAGGAGCTGGGCAAGGGGTG
AGATGAAGACCCTGACAATTTTAAATGACTGTAGCATAGAGAGCCATGGCCTTTGAG
TTTAAGAGTCTTGATCCCAGGTTCTGTCCCCCACTGTCCTGTGACTTAGCCACCTTGT
CTTGCTACAGATGGTGGTAGGAGGCCACCCTGTTGCGAAGTCCTGAGATAATGACAA
ACACAGAGGCTAGCTCACAAAAATGTACTTCCTGGCCTGGCTTCTGAAGGGTTAACT
GTTGGGCTCCATCCCAGATTTCTGAGATCAGGAACTCCAAATATGAGGCCCGCCTCT
GGCTGATTCTGATGCCCCATAAATGTTTGAAAATGACACAGCAAAGGTTCATCTCCA
GCCAGGTGTGGTGGGACACACCTGTAAGGCCAGCGCTTGGAGATGGAGACAGGGGG
ACCAGTAGTTCAGGGTCATTCTTGGCTACATAGCAAACTCAAGGCCACCCTGGTCTC
AAAAACCAAAACAAAAAGCCATCTTCTGACTCCCTTCAATTGTTCAAAGCCTTTCCA
GGGCCTTCAGAATCACGCTCAGAGTGTTCTGGGAAGATTAGCCCAGAAGCCAGAGA
AAGAGTACGCTGTGTGCTTGTAAAGCCAGTTACTCTGTCCCCTGTGAACTAGGAGAC
AGAGCACTTCCGACCCTATAGAGGGCAGTAGTGGCCATTCCTTGTAGGGGACTGGTA
TAGAAGTAATGTGAACTATTTAAAAATAGTTATTTAATTGCTGCCTTCACATTTGATT
TTATTTAACCTTCACATTATTTAGAAAATAATAAGAGTAGTAAGTGTCTGAATAGGA
AGGGAGTCTCTTAAGGCTCTTTCCAAGAGCTCAGGTTTGGATTTCTAGAGTCCCCCC
GACCCCAGAGAGGACTCTTTAGTGTTTGACACGGTCTTTGTAAGTAAGATGGGGAGT
CCTGGAGAGAGAGACCAAGCTGATTTTTAAACTAGGAAATGGAGTCTTGAACTGTG
GAAGATTTGAAAAGTTAAGCCTATGTGTCTTGAAGGTACTTGGCCAGAAAAGCACTT
GGCTTGAAAAAGAAAACCTGTTTAATTCAGGGGTGGAGGAATAGAGACAGATGAAG
AAAGCATTTAGACCTCGGAAACCTGATGTCCTATGAAATTCTGTTTTTATAAAATTGT
GTTATGGTGGAGATCTGTTGCATTTCGACTTTGTGGCTGTAAGAAACCTGTTATCTAT
GTTTAAGAAAGTACTTCTAATTTATTCAATGTCTTCCTAAATTATCCTTTAAAAAAAA
AAGTTGGAAAGTCTATGAGACCGTACCTAAGAAACCTTGACTGTGTATTTAAGTTAT
TTAATGCCATGCATTTGTGAAGCCCCTTCCCAGTGATGGCTGTGGTGTGTCTGAGGA
AATGTAAGTTTGGCATGAGGGGGAGGGGCTGCTGTTTCTATATTTGTTTTTGTTTTCT
ATAAACAGTAATCAGGATGTATCCTGGTTTCATTTGACATTGAAAAAAAAAAAAAA

ACTCGTGCCGAATTC-3' (SEQ ID NO:28). The L111-5 clone contained 0.5 kb of the 3'-end of an ORF.

Northern blot analysis using a sequence from L111 revealed the presence of a 4.0 kb mRNA transcript. This analysis also revealed that the expression of L111 mRNA was marginally upregulated in response to the multiple MECS treatment.

Another nucleic acid clone was designated L117. The L117 clone is 2460 bp in length and has a nucleic acid sequence as follows: 5'-TACGGCTGCGAGAAGACGACAG
AAGGGGAGCGGAGCCAAGATGGCGGCGGAGCTGGAATACGAGTCTGTGCTGTGTGT
GAAGCCCGACGTCAGCGTCTACCGGATTCCGCCGCGGGCCTCCAACCGCGGTTACAG
GGCATCTGACTGGAAGCTAGACCAGCCTGATTGGACTGGTCGCCTCCGAATCACTTC
AAAAGGGAAGATTGCCTACATCAAACTGGAAGATAAAGTTTCAGGGGAGCTCTTCG
CTCAGGCGCCAGTAGAGCAGTACCCTGGGATTGCTGTGGAGACTGTGGCCGACTCCA
GCCGCTACTTTGTGATCAGGATCCAGGATGGCACCGGGCGCAGTGCGTTTATTGGCA
TCGGCTTCACGGACCGGGGAGATGCCTTCGACTTTAATGTCTCCCTGCAAGATCACT
TCAAGTGGGTAAAGCAGGAAACCGAGATCTCCAAAGAATCGCAGGAAATGGATAGT
CGTCCCAAGTTGGATTTAGGCTTCAAGGAAGGGCAAACCATCAAGCTGAGTATTGG
GAACATTACAGCCAAGAAAGGGGGTACTTCTAAGCCCCGGGCCTCAGGAACGGGGG
GCCTGAGCTTACTCCCACCTCCTCCTGGAGGCAAAGTCACTATCCCCCCACCGTCCTC
CTCCGTTGCCATCAGCAACCACGTCACCCCACCACCCATTCCAAAATCTAACCATGG
AAGTAATGATTCAGATATCCTGTTAGATTTGGATTCTCCAGCTCCTGTCCCGACTTCA
GCACCAGCTCCAGCTCCAGCTTCTACAAGCAATGACTTGTGGGGAGACTTTAGCACT
GCATCCAGCTCTGTTCCAAACCAGGCACCACAGCCATCTAACTGGGTCCAGTTTTGA
GTCGCATTGGCAAGAAGTTGAGGACACTTGAAGAATAAAAATGACCTCAAGGGCAC
CATTCTATGAGGGAGTTGAGGGACGGCTTAATTTCCCAGGACCCAAATCAGTGGTCA
GTCTTTCCTGTAGCTTCTCTGTGCATTCAGGCTGGATTTTTTTTTTTTTTTTTTGGTT
ACCTCTGTGTTACTTGCTGTATATCCAGGAGACAATCTGCTGTTTCCTGCTCAGAACC
AAGCAAGGGAGTAGTGGGTATTATCACACTGACTGACTTTGCAGAGTTCAGAAGGC
CAACTTGATGAGTGGGAGTGACCTCGAACGTATGTAAATCCTTGAACTTATTTCAGA

ATCATCTCATGATTCCCTAGTTAGCAATTTCAGGAGAGACAAATGCCTTGAAACTGT
CTTCTCCACTAATCCGAGACTAAATATGGTCAGGCTGGCCCCAGGACTCATGAAGTT
AGGGTTTTCATGGGGGTAGATTTGGAGAAAGCTGTGTCCCGGCTCTCTTCTGTAAGG
CCTCCTTCAGGCTTACCCCATGCAGTGAACTTCCCGTGCTGGGTGGAGCCCCATCAC
CTTCTTGTGTGTTTACATGTTGTTTCCTTTGACAAGAGGGTTATGTTGGTGGCACCTC
ACTGTTTTCTTGTTGAATAGTGCAGCATCTTTGACCAGTGAATATTTCTGAGATGAAG
GGGTCAAGGGGCTGTGCTTTCCATGGTGTAGTCTACAGAAGTGTTTAATTTCTTGCG
GCCCCACGGGATTGCTGCACTGACGCATAGAATTGATCTATACTCACCCTGTGTTTG
ACCTGAAGAGTTTTAACTTGATGTGTAGAGCAGAGAGCTGGAAGCACTAAGTTCCCA
TTCAGTACCCACAATGCCTTGCTGCCTGGTTTGACTCCTTTTCATAAACATTTCATTT
CAGTCCATCTAGCACTTCTGTGGAAAGCTGCTGTTGATTGTGTCAGTGTGAAGGAGG
TGAAGTCACAGCTTTCTTTACCTATGACAGTTAGGCTTTGCACTAGACGTTGATACCA
GCTAGGATATCTTAAAGGAAGTTACCGCCCCATCACTCTCCAGTCTCTGGCCGCCAT
TCCTTTTACAGTGCTGTGAAGAGCGTCCTCTGAGGTCGGTGGGTACTGTCTCCTGTTG
GTCGGGCAGTTTGAGGGAGGAGTGGGAGGACTCACACTCCTGCAGGTACCTGTTTG
GGTAGCACACTGGCTGCAGAGAGTCCTTTCAGATATATTGTTTCTCAATGTTCTTCGT
AGCTTTTTCTAACTTCGGGTCCATTTTTCCCATCGCCTCTTCCCATTCCCAGGCAGCTC
TCTTGTTGCAGAGCCATGGCAGGACGTTTAAGTTCCAATAAAAACACTAAGAAGAA
AGTATAGAATCACTAGTGACTGTTGGGAAACCTATTTTCTCAATCTTCCTCCATTTTG
TGTTCTTTGTATTCTTAAGATGATAATATATTATGTATTTGAATTGCTGAAAATTGAA
AATGAAGTTGAAGATATATGTATATAAGCGTATGCTGTATTGGTGCAATAATGGTAA
TTAAAGATATTAAAAAAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3'
(SEQ ID NO:29). In addition, the L117 clone contains an open reading frame (ORF) from basepair 42 through basepair 875. This ORF encodes a polypeptide of 278 amino acid residues. The amino acid sequence of the L117 polypeptide is as follows: MAAELEYESVLCVKPDV
SVYRIPPRASNRGYRASDWKLDQPDWTGRLRITSKGKIAYIKLEDKVSGELFAQAPVEQ
YPGIAVETVADSSRYFVIRIQDGTGRSAFIGIGFTDRGDAFDFNVSLQDHFKWVKQETEIS

KESQEMDSRPKLDLGFKEGQTIKLSIGNITAKKGGTSKPRASGTGGLSLLPPPPGGKVTIP PPSSSVAISNHVTPPPIPKSNHGSNDSDILLDLDSPAPVPTSAPAPAPASTSNDLWGDFSTA SSSVPNQAPQPSNWVQF (SEQ ID NO:30).

Using tblast2x algorithms, the L117 polypeptide was found to have homology with expressed sequence tags (ESTs) from mouse, mouse embryo, human hNT neurons, human tumors, drosophilia, drosophilia embryo, *C. elegans*, and *Arabidopsis thaliana*, a plant organism. Although the sequence of ESTs can be questionable, the identified ESTs were aligned for comparison. The comparison of consensus sequences from each species provided evidence that the L117 clone or a L117 motif has a very strong pressure for conservation during evolution since it is conserved in a variety of very distant species. In addition, this alignment indicated that the first methionine residue in the ORF of the L117 clone is the true initiation site for translation since most of the homology between the ESTs begins around this position, and the *C. elegans*, drosophilia, and human hNT ESTs each contain a methionine residue that is in a very close proximity to that of the L117 clone. Further, the relation between these ESTs and the L117 clone was supported by an exactly matching stop codon in the human EST, mouse EST, and L117.

Northern blot analysis revealed that the expression of the L117 mRNA was not upregulated in response to the multiple MECS treatment in either the hippocampus or cortex. Analysis using a total RNA extract, however, revealed a small upregulation upon MECS stimulus.

Another IEG nucleic acid clone was designated L119. The L119 clone is 2900 bp in length and has a nucleic acid sequence as follows: 5'-ATTCGGCACGAGCCAGAG TGAAGGGGCATGGAGAAGTGGACGGCCTGGGAGCCGCAGGGCGCCGATGCGCTGCG GCGCTTTCAAGGGTTGCTGCTGGACCGCCGCGGCCGGCTGCACTGCCAAGTGTTGCG CCTGCGCGAAGTGGCCCGGAGGCTCGAGCGTCTACGGAGGCGCTCCTTGGCAGCCA ACGTAGCTGGCAGCTCTCTGAGCGCTGCTGGCGCCCTAGCAGCCATCGTGGGGTTAT CACTCAGCCCGGTCACCCTGGGAGCCTCGCTCGTGGCGTCCGCCGTGGGCTTAGGGG TGGCCACCGCCGGAGGGGCAGTCACCATCACGTCCGACCTCTCTCTGATCTTCTGCA

ATTCCCGGGAGGTACGGAGGGTGCAAGAGATCGCCGCCACCTGCCAGGACCAGATG
CGCGAACTCCTGAGCTGCCTTGAGTTCTTCTGTCAGTGGCAGGGGCGCGGGGACCGC
CAGCTGCTGCAGAGCGGGAGGGACGCCTCCATGGCTCTTTACAACTCTGTCTACTTC
ATCGTCTTCTTCGGCTCGCGTGGCTTCCTCATCCCCAGGCGTGCGGAGGGGGCCACC
AAAGTCAGCCAGGCCGTGCTGAAGGCCAAGATTCAGAAACTGTCTGAGAGCCTGGA
GTCCTGCACTGGTGCCCTGGATGAACTTAGTGAGCAGCTGGAATCCCGGGTCCAGCT
CTGTACCAAGGCCGGCCGTGGTCACAACCTCAGGAACTCCCCTGATCTGGATGCAGC
GTTGTTTTTCTAAGAGCATCCTCTAGCTGTGTGGAATGTTCTAGATTCGCAGCATCCA
CAAGGAAGTGCTACATGGGCGGAGTGCAAAGGATTTCAGAAGCTCTTCTTGCAGGG
CATCAGTCCGTAGCTCCTTGTGTGTGCGAAAGACTTTTCACTTGTGTAATCCCAACTG
AGTATGTGACCCTAAACAGTCACTTTGGGGACTCCCCAAATCCTTTTTAGCTGCACA
CAGCTTGTCAGACTGTCCTTCAATTAGAGTTATTGGGGTGGGGGGGCTTGATGGCTT
GAGTAATAGAGGTCTGGCGAGGTGTCTCCCTCTTGGACCTCTTATGTGTTGTTACTAG
AATCCTGAGATTCTCAAATGTTGGTGAGAGGAGACTTTTACTTTTCAACTTTGCTTCG
GCAGTTTCCGATACACAGGACTCCAGAATCCAGAACAAGAAAGAAGAACCTTGTGT
TTGTAGGGTGTGCAGACCCAGACGGGGCCGAGGAGCTGACTTGCTCAGCTCTCACAC
GCAGCCAGTTTATCCACTCACAGACCAAACCTGGCTACTGCATAGACTGTTCCAGTG
TGGCTTCAAATCCACACCTCTAGGTACCCTGAGAAGGAAAGCCACCTGAAGAGTCA
CTCTAATCCCAACACGCTCACCCCCTTCACGTCCATAAAGGAGCTGGGCAAGGGGTG
AGATGAAGACCCTGACAATTTTAAATGACTGTAGCATAGAGAGCCATGGCCTTTGAG
TTTAAGAGTCTTGATCCCAGGTTCTGTCCCCCACTGTCCTGTGACTTAGCCACCTTGT
CTTGCTACAGATGGTGGTAGGAGGCCACCCTGTTGCGAAGTCCTGAGATAATGACAA
ACACAGAGGCTAGCTCACAAAAATGTACTTCCTGGCCTGGCTTCTGAAGGGTTAACT
GTTGGGCTCCATCCCAGATTTCTGAGATCAGGAACTCCAAATATGAGGCCCGCCTCT
GGCTGATTCTGATGCCCCATAAATGTTTGAAAATGACACAGCAAAGGTTCATCTCCA
GCCAGGTGTGGTGGGACACACCTGTAAGGCCAGCGCTTGGAGATGGAGACAGGGGG
ACCAGTAGTTCAGGGTCATTCTTGGCTACATAGCAAACTCAAGGCCACCCTGGTCTC
AAAAACCAAAACAAAAAGCCATCTTCTGACTCCCTTCAATTGTTCAAAGCCTTTCCA

GGGCCTTCAGAATCACGCTCAGAGTGTTCTGGGAAGATTAGCCCAGAAGCCAGAGA
AAGAGTACGCTGTGTGCTTGTAAAGCCAGTTACTCTGTCCCCTGTGAACTAGGAGAC
AGAGCACTTCCGACCCTATAGAGGGCAGTAGTGGCCATTCCTTGTAGGGGACTGGTA
TAGAAGTAATGTGAACTATTTAAAAATAGTTATTTAATTGCTGCCTTCACATTTGATT
TTATTTAACCTTCACATTATTTAGAAAATAATAAGAGTAGTAAGTGTCTGAATAGGA
AGGGAGTCTCTTAAGGCTCTTTCCAAGAGCTCAGGTTTGGATTTCTAGAGTCCCCCC
GACCCCAGAGAGGACTCTTTAGTGTTTGACACGGTCTTTGTAAGTAAGATGGGGAGT
CCTGGAGAGAGAGACCAAGCTGATTTTTAAACTAGGAAATGGAGTCTTGAACTGTG
GAAGATTTGAAAAGTTAAGCCTATGTGTCTTGAAGGTACTTGGCCAGAAAAGCACTT
GGCTTGAAAAAGAAAACCTGTTTAATTCAGGGGTGGAGGAATAGAGACAGATGAAG
AAAGCATTTAGACCTCGGAAACCTGATGTCCTATGAAATTCTGTTTTTATAAAATTGT
GTTATGGTGGAGATCTGTTGCATTTCGACTTTGTGGCTGTAAGAAACCTGTTATCTAT
GTTTAAGAAAGTACTTCTAATTTATTCAATGTCTTCCTAAATTATCCTTTAAAAAAAA
AAGTTGGAAAGTCTATGAGACCGTACCTAAGAAACCTTGACTGTGTATTTAAGTTAT
TTAATGCCATGCATTTGTGAAGCCCCTTCCCAGTGATGGCTGTGGTGTGTCTGAGGA
AATGTAAGTTTGGCATGAGGGGGAGGGGCTGCTGTTTCTATATTTGTTTTTGTTTTCT
ATAAACAGTAATCAGGATGTATCCTGGTTTCATTTGACATTGAAAAAAAAAAAAAA
A-3' (SEQ ID NO:31). In addition, the L119 clone contains an ORF from basepair 28 through basepair 768. This ORF encodes a polypeptide of 247 amino acid residues. The translational start site was assigned to the first methionine residue in the ORF. The amino acid sequence of the L119 polypeptide is as follows: MEKWTAWEPQGADALRRFQGLLLDRRGRLH
CQVLRLREVARRLERLRRRSLAANVAGSSLSAAGALAAIVGLSLSPVTLGASLVASAVG
LGVATAGGAVTITSDLSLIFCNSREVRRVQEIAATCQDQMRELLSCLEFFCQWQGRGDR
QLLQSGRDASMALYNSVYFIVFFGSRGFLIPRRAEGATKVSQAVLKAKIQKLSESLESCT
GALDELSEQLESRVQLCTKAGRGHNLRNSPDL DAALFF (SEQ ID NO:32). Hydropathy plot analysis revealed a stretch of about 50 hydrophobic amino acid residues, possibly indicating that the L119 polypeptide is a type II transmembrane protein.

Northern blot analysis using a sequence from the L119 clone revealed that the expression of the L119 mRNA was strongly upregulated in response to the multiple MECS treatment. Specifically, L119 mRNA expression was induced 17.8 fold by the multiple MECS treatment as determined from Northern blot data using total RNA from rat hippocampus (Table I).

Another IEG nucleic acid clone was designated R010. The R010 clone is 1280 bp in length and has the following nucleic acid sequence: 5'-GCTTTGGAAACCGGACTGCAGGCT
AAACTGGCTTCTTTTGAATCCTTGGAAGCATAAAGGACAAGTAGCAGGGCTCGCAGT
CTTCCATTTGTCACTGGAGAAGAACTTATAATTCAGAAGATCTGGGTCTGGACCCAG
GCTGACCACTTTGGAGCTTTGAGACTCTGGGATTGTGATCCAGTTCTGAGCTGGTGA
TAAACACTCCTTGTGACTTTTGGTCAATTCAGCTACCAGATTCCAGCCAACATGACC
CTCGCAGCCTATAAGGAGAAGATGAAGGAACTCCCACTAGTGTCTCTGTTCTGCTCC
TGTTTTCTGTCTGATCCCCTGAATAAATCATCCTACAAATATGAAGGCTGGTGTGGG
AGACAGTGTAGGAGGAAAGGTCAAAGCCAGCGGAAAGGCAGTGCTGACTGGAGAG
AAAGAAGAGAACAGGCAGATACGGTAGACCTGAACTGGTGTGTCATCTCTGATATG
GAAGTCATCGAGCTGAATAAGTGTACCTCGGGCCAGTCCTTTGAAGTCATCCTGAAG
CCACCTTCCTTTGACGGGGTGCCTGAGTTTAATGCCTCCCTCCCAAGACGTCGAGAC
CCATCGCTAGAAGAGATACAGAAGAAGCTAGAAGCAGCAGAGGAGCGAAGGAAGT
ACCAGGAAGCTGAGCTCCTAAAACACCTTGCAGAGAAACGAGAGCATGAGCGTGAG
GTAATCCAGAAAGCTATCGAGGAAAACAACAACTTCATCAAGATGGCGAAAGAGAA
GCTGGCCCAGAAGATGGAGTCCAATAAGGAAAACCGGGAGGCCCATCTGGCTGCCA
TGTTGGAGCGGCTGCAAGAGAAGGACAAGCACGCAGAGGAGGTGCGGAAAAACAA
GGAGCTGAAGGAAGAGGCCTCCAGGTAAAGCCCANAGGCCAAGGAAGTTTCCAGGA
CAGCCGGACAGCTCCCGCAGCAACCTGGTTCCAGCAGCATCGGCCGCTGGCTGCTCT
CCCAGCACTGGGGTTCGGGGGGAGGGGGGTGGCCAAAGGGGCGTTTCCTCTGCTTTT
GGTGTTTGTACATGTAAAAGATTGACCAGTGAAGCCATCCTATTTGTTTCTGGGGAA
CAATGATGGGGTGGGAGAGGGGACAGAGAGTGTTTGGAAAAGGAGGTGAAGATGA
GCCCGAGGACTTTGTGACACTGTCCACTGACTGCAGACTTGGGCCAAGGCCCCCGCT
TTTCACGGCTCTGCCTGGACATTCGGCCTCCAGGTTCCTAGTGGAGAGAAGATGTGA

CAGAAGTTCAGAGTGAAGGGCCGAGTCCTGGTGGGGTGGTGTGCAGGGCCAGCAGG
ACGAGCCCGTCTGGATGGAGTGAAACCTACCCTGAGCGGGTGGGATAAGGTCTGTG
TGCGTCTGTTCATTGTCATCTTTTGATCATCATGACCAACGAAACATTTAAAAAAAA
AAAAAAAAAAAAAAA-3' (SEQ ID NO:33). Two genomic R010 clones were also obtained.
The nucleic acid sequence for these genomic R010 clones is as follows: 5'-GATAA
ACACTCCTTGTGACTTTTGGTCAATTCAGCTACCAGATTCCAGCCAACATGACCCTCG
CAGGTAGGTACATGCACCAGTCAGTGATGAACACCATAACACAAGCCATTTTTCTAT
CTCTGTGTGTGTCCATGTGTATTAAGGTGCATCCGTGTGTGTGATACACACGTAGGT
GCATGGCATGCATGTGTGTGCAAATGCATATACAAGTCCAAGGACAGGGGTTGGGG
ATTTAGCTCANTGGTAGAGCACTTGCCTANGAAGCGCAAGGCCCTGGGTTCGGTCCC
CAGCTCCGAAAAAAAGAACCAAAAAAAAAAAAAAAAAAAAAAAAAAATTTCCAN
GGACAACCCCAAATTTCCTTTCNCNAAANCCANCCANCTTCCATTNAAAAAAAANG
GGTCNCNCNTGGGTTAAACCATTTNNAAANGGCNAACCTNACNGGCCAKTGAKTGC
CAGGAATCTTCTTATYCCTGCCCWACCTCCAATGTCTTTCACATGTGAATGCTGAGG
GTCAGAACTTGTGCTTACAAGGCAGACATTTTGCCAGCTCTCCGGCCATCTTTCTCTA
TGTATGTACACTCACAGATGCACAGGAAGAGAGGGTAGAGAAGCCAAGAGGCAAA
GTCATTTCTGGGTGGTGGGTGGGATCACAGCTGAATTCTTCTTCCTCATTTGCTCTGT
GTGTATTATTTAATTTTAAAATAATACCTTTATAATAGTATCGAAACTATGCTTTCAA
GTTTGTAAGAGAAAGTGATCACTGGGCTGTGTAGTGAGGGGGTCTTTATATTATGCA
TATAACATGGTGCAATGGGAAGGACTGGCAGAGGCCTCCATGATGACCTATGACTTC
TAGGGAGACTCAGTCGTGTCAAGGGTACATTCCTACTCTGCAGACAGCTTCTCCCTG
GTTTGATTCCTGTGCTGGGAAGATTTGAGGAGTCTTCCAGCCTGACCTCTTCTACAGT
GGGCCTGGACTTTAAGGAGAGTAGCAAGGAAGTCTTTTTATTAATCTCTTACCCTTT
AGGCAGCAGTGTCAAGTACTTTTAGCAGAATTAAATATAGATTTCCTACAAACTACA
AACTTCAAAGCCCTGGTTTATCCTTGGGTGGGAGTAGGAGATGGAGGGCCAGGGTC
AGGGCACTGCACTTGGGATCTTTACTTGAGGGTACTCAACGCTTGGTAGTAACAAAA
AGTGGGGTGAGTGACAATGTTAATTTTCAACTGGGAGGTAGCCCAGGCTTGGGTACT
TTGGAGCCAGAAAGCCTGGGCTGACTCACAGAAGTGGTGCTCTCTCTYGYAGCCTAT

AAGGAGWWGATGAAGGAACTCCCACTAGTGTCTCTGTTCTGCTCCTGTTTTCTGTCT
GATCCCCYGRATAAATCATCCTACAAATATGAAGGTGAGTAGGGGCTAGGCTGGGA
TAGAAAAGGGTGGAGGCTTCTGTGTCCTGTGTTTGTSGGTGCCCCACATTGACTCCTA
TCTTGTAAAACTGTCCTGGTCGCAGTGTGTCTTATTTCCCAGAGGCTGAGGAGTCTG
AGCCCAGGGGGATGTAGCCTGGGTGCCAAGCAGCCTCCAGGGATCTGGATTGGGCC
CTCCTGGAGCACTTGCTCCTAGAGTCCCTTTTRCACATTCCTTGACACCACAGAGGAC
ACCAGGATAAGCCAGACACAAGTTTTGAGATTCCATTCATGGAGGCCCAGAACAGA
AAAAGAAAACTTAGTGTGTTCACCAGGGCTTCTAGGGACAGGTAGAGATGCTCCTA
GACAGGTCCAGGGTGGGAATAGCACTTCTAACCTGGATGGTGACAGTTCGAGCCCCT
AGACCCTATCAGAGAGTACTGGATTGTCATGCTGTCAGGAGGAGTGGTCAGGGGAC
AGATAGGTCATCTCTTCATTTCTGTTTGCCAGGAAGGGATGGGTTTGGTCTGTCAATA
AGAGAGATGGGTGTTTGGATGACCTGAGTCTGTTTTTTCCATTTAGGCTGGTGTGGG
AGACAGTGTAGGAGGAAAGGTCAAAGCCAGCGGAAAGGCAGTGCTGACTGGAGAG
AAAGAAGAGAACAGGGTAGGCCGGAGCCAGGGGAGAGGTCCACAAGCCATCAGAG
GGACAGGGCAAGGAGGGGCTGGCGGTGGGGATGGGTGAAATGAACTGGTGTCTGTC
ACCAGCGAGGAACAACAGCAGCTGGTGCTATCACAAATCACAGCTCCCTGCTTACCC
TGTAAAAGCCATTGACCTTAGGGTCCAACGTTCAGGATCGACCAGACCCCTAGTCAT
TGGTGTGCCTTGGGACCCTCAGCTTTCCTGTGTCTGTGTGCATGTACACATGCTCATT
GGGGCCCCAGCTGCTCCTCAGAAGGTGAGCAGCCCCAACTCTGCCCTCCATAGCAGA
TACGGTAGACCTGAACTGGTGTGTCATCTCTGATATGGAAGTCATCGAGCTGAGTAA
GTGTACCTCGGGCCAGTCCTTTGAAGTCATCCTGAAGCCACCTTCCTTTGACGGGGT
GCCTGAGTTTAAAGCCTCCCTCCCAAGACGTCGAGACCCATCGCTAGAAGAGATACA
GAAGAAGCTAGAAGCAGCAGAGGAGCGAAGGAAGGTTAGTGTAGCCCCATGTCACT
TCCTCCCATCCCAGCGGGAGCAGGAAGTGCAGCTCCATATCTCTTCCTCCCATCCCA
GTGGGAGTGGGAAGGATATTTAGACAGCACCTCCTGAGTGCTGGGCATAGACCGGT
AGTTCTCAACCTTCTTAGTGCTGTAACCCTTAATATATATATATATATATATATATAT
ATATATATATATAGTTCCTCATGTTGTGATTACCCCCCATACCATAAACTTATCCCGT
TGCTCTTTATGTCTTCATAATTATAATTTTGCTACTGTTATGAATTGTGATACAACTAT

CAGACCTGCACCCCCTAATGGCAGCAGCCCACGTGTTGAGAACCACTGGCATAGAT
GTAGACTAAGATACCACCTGAAGGGGACAAGACTATGACTATGCACTGGGTGAGCT
TACAGTGTGGCTAATGGCTAAAATGTCACAGTCCTCACAAAGCTGCCTTTGTATGCA
GCTTCCTTGTTCCCCATTGATTCTMGTCCSTCAGCTCAGATGCCCATTTTAATGTGAG
TGTTTCTTNACCTTTCAGAAANACAAAACAAAACAACCCAGCTTTCTCCACTNAATT
GTGTGGTCCCTCCCTTTAAATATCCAAAGCATTTATCACACCCAGGTCTGGNGTCCA
NTATNTATTGATATGCGTGTTTATTTNNACTAGGGCAATTNTCTCCNTTCCCTGGTGT
CTGGAGTTGTGAGGGCCTTGAGGTTTATAGAAGATCACTTAGTACTTGTGAATGAAC
GCGAGGAAAAGGAGAAAAGAGACTCAGAAGCTACTTNGGAAAGGGCTACNAAAGC
CAAATATGACGGAAAGGTTTGCAGTCCATGNCGTTGTTCTCTGCTTCTGGGACAGAG
GACCAGGTTCATCTCATCTGGGCATGGCACTGTTCAGCTGTGGTGGTAGAAATCCAC
TCTAAAGGGTCNTTCTCTTTCTTTTGNTGCCCTAGTACCAGGAAGCTGAGCTCCTAAA
ACACCTTGCAGAGAAACGAGAGCATGAGCGTGAGGTAATCCAGAAAGCTATCGAGG
AAAACAACAACTTCATCAAGATGGCGAAAGAGAAGCTGGCCCAGAAGATGGAGTCC
AATAAGGAAAACCGGGAGGCCCATCTGGCTGCCATGTTGGAGCGGCTGCAAGAGAA
GGTAAGAGGTCCTGGATTGGCAGGAGGCTCCTTCCATGGCAAGAACGTGCAACCTA
CACATCACTCTGGAGGAAGCGGCCTATGCAGGAATTGAAATGTTTCTACCAGGCAG
GGTCCTCATTGTTCTAAGGGGAAGATTTGGGAAGTCATAGGCAAGAAGCTCACACC
AAACCCTGGGTGGCCTCCGGGGATCTTCTANGGTTTTGAACCGGAAATTCTGCACTG
TCTCANGAGCTTGCTCACACCCTTCTTTTCTAAAGAAAGCCCGCCCAGTGCAAGTAT
CTAAGGAGAGGCACATGTCTACACATTTCTGGCTTCATCATTGAATGGGCAGATTTG
GGTTAGTGAAAGATACAGTCAGCTTGGCTTTGAGCCANGGATACAGCAAGCTCGGTT
GCCAATACAGCAGGATACAGGATTCTCCCCAGAGCTCCTCGTAAGGGCCAGAGAGT
ANTAGGTTTTCCTCAATAGTCTGCCTTTGTCAATAACTCAAATGTCACCTGCATCTGA
GCGGTGTGCGAGACTGGGGTTGGTCCTCCATGTTATTCTTTGGAAGACGTGCTGACC
TCATTTCCTGAGTCCCAGGCTGCCTACGTTTCTCCTGCAGCTCCTGGGAAGCTTTAGC
TCTGTGTTTTATTTCCAAGGAGCCGCCTGCTGCGCGGTGACTCCCGGGACSGATCGGT
GGCCTCGTCCCATGGTGAGCAGCGTGGTCCTTATTCCTTCCTGCCTACCCACCTAAAA

CCTCAGGCCCTTGACAATTACCACAGAAAGATCTGGCTTCATCCAGGGATGTGAGCA
GCACAGGCTGGCCAGTAGGTGGCAGCCCTGTGCTCATGTTCAATTACAGGAGGGAC
AGCAAGGCTTCTTTCTCCACTGAGTGCCTTGGGGGAGGGACACAATCTGAGTGTGAC
TTTGGGCTCCTCCAGTTAATGAGAGATACTGTAAGAAAACTTAAGATTGCCTTTACT
TTTTATACCAGGTCTCATGCATTCCAGGCTGGCCTCAAATTGGCTAAATTGCTGAGG
CTAGCCTTGGAATTCTTATCATTTTGTCTTCACCTCCAAGTGCAGGGATTACAGGCAT
GTGCTGCCAAGCCTATTCAATGCAGGTTTGGGGCTTGAACCCAGGGCTCTGTGCATG
CAAGCTAGGCACTCTGCCAACAGTGCCATAGCCCCAACTCAAGGCAAATTCTTGAGG
AAACCACAGATAGAATGGGAGAGTTATGGGATTGCAGACTCAGCTTAAAATACATC
ACAAAGTTAGGTTGTGTTGAAGCACTTGAATGTTTGTTTATATAACGATTCTATTTTA
TCATAACTCGGTCATCACAAGTTTACAAGGCAAACATTCTTAGTCCAGATAAGGAAA
CCATTCTAGAGGTCAAATGATTCCAGAGATTNACAGGGTATACGACAATANATTGGC
CCTGGCCNCTAATCAATGGCTGCTTCTTGCCGGGTAAAGAAAACATCCAATATAANC
CACNNCTTTCANAGCAANAATTTCAAAGACAACAAGCAGGGCAAAACCAGGGTCCA
AAGCAACCACT-3' (SEQ ID NO:34) and 5'-TGGGCGGGAAAGCAG
TTTGTCTTGTTGNTGAATTATGTTANNAAGCAAATGAAGTTATCTTCCAACACATGTG
AGGGAGTCCATTGTCTGGAGTCAAGCANTATTTCCCAACAGTTCTCTGTCAGTACAT
AACGCAAGGTCCTCCTTCAGTCAGAGATTTAAGACAACACTAAAGAGATGGAGAGA
AATAACACATCTGTGGTGTGTCAGGGACGCTGGCAATGGGCTGATCTTTTCCCATTC
NTTNTAAACTGGCTGTCCCAAAGGGCCCNTTGTATTTAGTCAAGTGACCATTCCAAG
CGCCAGAATGACCAGTGGAGGTGCAGAGAGCNTAGGGTGTCTTGGGGTCGCTGTGA
GGTGGGTCCCCTGCAGGATGTCTATGCACTTGCAGGCTTATACACCTGTGTCCCGCG
TNTTACTTGCCTCCTTCCACCCCTCTTAGGATACCTTCGCCGACAGCTCTGCTCTGCC
CGTGGTGACCATCTTTTGCGCTCCATTCTCTTGCCCTTTGTCTTCCCCTGGCAGCCTTG
TGTGACCCGCCTTTGTCCCTCCCTTCCTCTCCAGGACAAGCACGCAGAGGAGGTGCG
GAAAAACAAGGAGCTGAAGGAAGAGGCCTCCAGGTAAAGCCCAGAGGCCAAGGAA
GTTTCCAGGACAGCCGGACAGCTCCCGCAGCAACCTGGTTCCAGCAGCATCGGCTGC
TGGCTGCTCTCCCAGCACTGGGGTTCGGGGGGAGGGGGGTGGCCAAAGGGGCGTTT

CCTCTGCTTTTGGTGTTTGTACATGTAAAAGATTGACCTGTGA-3' (SEQ ID NO:35). In addition, the R010 clone contains an ORF from basepair 80 through basepair 727. This ORF encodes a polypeptide of 216 amino acid residues. The translational start site was assigned to the first methionine residue in the ORF. The amino acid sequence of the R010 polypeptide is as follows: MTLAAYKEKMKELPLVSLFCSCFLSDPLNKSSYKYEGWCGRQCRR KGQSQRKGSADWRERREQADTVDLNWCVISDMEVIELNKCTSGQSFEVILKPPSFDGVP EFNASLPRRRDPSLEEIQKKLEAAEERRKYQEAELLKHLAEKREHEREVIQKAIEENNNFI KMAKEKLAQKMESNK ENREAHLAAMLERLQEKDKHAEEVRKNKELKEEASR (SEQ ID NO:36). The R010 clone was found to have homology to the stathmin family of polypeptide, including stathmin, SCG10, and XB-3. In addition, the R010 polypeptide was found to contain a unique 27 amino acid sequence (encoded by exon 3) that is alternatively spliced to lead to the formation of two distinct mRNA transcripts.

Northern blot analysis using a sequence from the R010 clone revealed that the expression of L119 mRNA was restricted to brain. In addition, R010 expression was found to be developmentally regulated. Further, R010 expression was found to be rapidly induced *in vivo* in the dentate gyrus in response to the multiple MECS treatment and LTP stimulation, and rapidly induced *in vitro* by NGF treatment of PC12 cells.

Another IEG nucleic acid clone was designated R042. The R042 clone is 3978 bp in length and has a nucleic acid sequence as follows: 5'-CGGCGATGGCGGCGGCTGCT GTGGTGGCAGCGACGGTCCCCGCGCAGTCGATGGGCGCGGACGGCGCGTCCTCCGT GCACTGGTTCCGCAAAGGACTACGGCTCCACGACAACCCCGCGCTGTTAGCTGCCGT GCGCGGGGCGCGCTGTGTGCGCTGCGTCTACATCCTCGACCCGTGGTTCGCGGCCTC CTCGTCAGTGGGCATCAACCGATGGAGGTTCCTACTGCAGTCTCTAGAAGATCTGGA CACAAGCTTAAGAAAGCTGAATTCCCGTCTGTTTGTAGTCCGGGGTCAGCCAGCTGA TGTGTTCCCAAGGCTTTTCAAGGAATGGGGGGTGACCCGCTTGACCTTTGAATATGA CTCCGAACCCTTTGGGAAAGAACGGGATGCAGCCATTATGAAGATGGCCAAGGAGG CGGGTGTGGAGGTGGTGACTGAGAACTCTCACACCCTTTATGACTTAGACAGAATCA TCGAACTGAATGGGCAGAAACCACCCCTTACCTACAAGCGCTTTCAGGCTCTCATCA

GCCGTATGGAGCTGCCCAAGAAGCCAGTGGGGGCTGTGAGCAGCCAGCATATGGAG
AACTGCAGAGCTGAGATCCAGGAGAACCATGATGACACCTATGGCGTGCCTTCCTTA
GAGGAACTGGGATTCCCCACAGAAGGACTTGGCCCAGCTGTTTGGCAAGGAGGAGA
GACAGAAGCTCTGGCCCGCCTGGATAAGCACTTGGAACGGAAGGCCTGGGTTGCCA
ACTATGAGAGACCTCGGATGAATGCCAATTCCTTGCTGGCCAGCCCCACAGGCCTCA
GCCCCTACCTGCGCTTTGGCTGCCTCTCCTGCCGCCTCTTCTACTACCGCCTGTGGGA
CTTGTACAGAAAGGTGAAGAGGAACAGCACACCCCCCCTCTCCTTATTTGGACAACT
CCTATGGCGAGAATTCTTCTATACAGCGGCCACCAACAACCCCAGGTTTGACCGAAT
GGAGGGGAACCCCATCTGCATCCAGATCCCCTGGGACCGCAACCCCGAAGCCCTGG
CCAAGTGGGCCGAGGGCAAGACAGGCTTCCCTTGGATTGACGCCATCATGACCCAA
CTGAGGCAGGAGGGCTGGATCCACCACCTGGCCCGGCACGCTGTGGCCTGCTTCCTC
ACCCGAGGGGACCTCTGGGTCAGCTGGGAGAGCGGGGTCCGGGTATTTGATGAGTT
GCTCCTGGATGCAGATTTCAGCGTGAATGCAGGCAGCTGGATGTGGCTGTCCTGCAG
TGCTTTCTTCCAACAGTTCTTCCACTGCTACTGCCCTGTGGGCTTTGGCCGACGCACG
GACCCCAGTGGGGACTACATCCGGCGATACCTGCCCAAACTGAAAGGCTTCCCCTCT
CGATATATCTATGAGCCCTGGAATGCTCCCGAGTCGGTTCAGAAGGCCGCTAAGTGC
ATCATTGGCGTGGACTACCCACGGCCCATCGTCAACCACGCAGAGACTAGTCGGCTC
AACATTGAGCGGATGAAGCAGATCTACCAACAGCTGTCACGATACCGGGGGCTCTG
TCTGTTGGCATCTGTCCCTTCCTGTGTAGAAGACCTCAGTCACCCTGTGGCAGAGCCT
GGTTCTAGCCAGGCTGGGAGCATCAGCAACACAGGCCCCAGACCACTGTCCAGTGG
CCCAGCCTCCCCCAAACGCAAGCTGGAAGCAGCTGAGGAACCTCCAGGTGAAGAAC
TGAGCAAGCGGGCTAGAGTGACAGTGACTCAGATGCCTGCCCAGGAGCCACCAAGC
AAGGACTCCTGAGACTGGAGAGCCATTGCTCCGTGAGCAAAGCCCAGGTGCCTGAG
CTGCCATGGCCACAGAGAAGACATGGAACCTACAGAGAAGACAGTCACCAACAGAC
AGAGCGAGCGACTGTGTGTGTGCAGAGGGAGGTGTGGTGTGCCGTTTGCGTGTGCAT
GCATCTGTTTACACTCTCATGATCCTGAATGTTGCCTGTGCTGGAGGAGCCCCTAGAT
CATGCCTTCTTACCAGGGCTGTTTCTTGACTTCCAGACATAAGACTAGAACCCGCAG
CAGTAACCGTCAGCCCAAATCTGCCCCTGGGAGCCCCAATAGGGTGGTAAGACCCT

AGCTTGAATTCTGGTCTCTGCCTCCCCAGACTCTTCTTCCTCCCTCCTTTTAACAAGG
AGCTGGAGGGCCACATTTTTGACTCTCATCTAAAGCATGGAGTTTCAGAGGCAGTCA
GAGTCCTGCTGACTTAGTTCCCACTTTTCTGACACTAGAACCTGAGCAGGCTGGAAT
AGATGTGTCCTGTTGATCTTAAACAGCCTGGCCAGTCTTCTTATAAAATCCTGTGCCA
TTAACAGGCTTCCCTGATGTCTAAGGCTACAGACTAGTGTGTTGTGTGCCCAGTACT
GCTTATGTCAGCCTCAGACATAATATCAGTCTTTGTAGAACCTTCTAAAAAAAACCA
CATGGGGAATAGACTCCCAGTCTTCTGTCCCTTCCCTAGCAGCTAAGGTCCAGTCTC
GACCTTCTAGAAGCTGTGGACAGGCTAGGGTCTGAACTGGTGAAAGAAACCCAGGT
CCCACAGCTGCAGGGCCCCTGGTTCCTCTGGCTGTACTCCTGACACCACATGCTCCA
GCCAGTACTGCTGATATCCAGCCAGGCAAGCTGGACAGCCTGGCTGGTCAGCACCTG
CCCTGCAGTGTCAGCTGCCCAGGACTGAGCTTCCGGAGACTCAGACAGACTTAGGG
GTGGAGCACTGCCTCTGGCAGTTGGCGAGAGGTCAGAGACCATGCCTGGCACATCA
ACATCTTCGCAGAGCAGCAGTGAAGGATTGACATAGAGAAGTCAAGCCTTGCTTTCC
AGGGGAGCCAACTCTCCCTCCCACTGTTGGGTCATATGGAGAAAGAAGTTATGAAA
GGATCTGGGGGTACCTGAGCAAGTCTTCCTTCCACCCCGTGGCCTGCATTTGAGCCA
CAGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT
GTGTGTGTAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
AGAGAGAGAGAGTTTGTTTCTGTTTGGATTTTTGTTCTCACATGTAACATTAAGCTGG
CCTCTGGGCCTTTTCCTCTCTACCTCCCCTGTGACCTTTCCTAGCCTCAGAGTTGTTAA
TGCCCTTGGCCCTGGCCTTTTTTTTGTGTCAGACCAGAACCCTGGGGTCAGGCTCCCC
CCTCCAGCTGTCTAGCACATCTGACAGGCTTCTTTTTGAGATGGCCTCAGGTTTTCTC
AGCAGAGAGCTGCCTTTAGTCCAACTGTTTATGTTCATCATCCTGACTAGAAGCATC
CTACGATTGTGTGAAGAAACGGCATCTGTGATGCCATGTTCAGAGTCATGGGGTGTG
GCCTCCCTGTCCCTAGCCCCAGGCCAAGAGGAAAGGGCCAAAGGCTCTTGCTGGAG
GGACAGTAGAATGCGTCTGGAGAACTGGTCCCAGAGGAGCAAAGGCTTATTCTGGG
GCCAGTATTTATTTTGCAACATCTTCAGCTATGGGGACAATGGCCTTCTCTGCTTTTT
TGATGATGGCTCTCTCCTCAAGGTACAAGTTGGCAAGGTCATCTGTCCTTCCACCTCC
TTGACATGTTGGCCCATTTCCAGGACAGCCTTCCAGTGAATGGAGCAGACTATTCCA

CAGCTGTGGGATAGAGTGTCTTGGAGCCCTGGAATGACTTCATGCCTCCTTTTGCCT
AGCCTGAGTGGCCCTGAGGACTGTCACAGAACAGTGCCCCATGTCCTGCTCCTGGGC
CCGAGCATGGGGAAGAGATGGTTGCAGGCAAGAGCACTTTACAGCATTCCCCATTG
CTGGGAAGGTTGTTTCTCCTACAGTGTGTGAATACTTACCTGTTTTATAAATGTCTGA
TCCTGTCTGAGTAAAAAAAAAAAAAAAAAAAAAAA-3' (SEQ ID NO:37). In addition, the R042 clone contains an ORF from basepair 51 through basepair 1790. This ORF encodes a polypeptide of 580 amino acid residues. The amino acid sequence of the R042 polypeptide is as follows: MGADGASSVHWFRKGLRLHDNPALLAAVRGARCVRCVY
ILDPWFAASSSVGINRWRFLLQSLEDLDTSLRKLNSRLFVVRGQPADVFPRLFKEWGVTR
LTFEYDSEPFGKERDAAIMKMAKEAGVEVVTENSHTLYDLDRIIELNGQKPPLTYKRFQ
ALISRMELPKKPVGAVSSQHMENCRAEIQENHDDTYGVPSLEELGFPTEGLGPAVWQGG
ETEALARLDKHLERKAWVANYERPRMNANSLLASPTGLSPYLRFGCLSCRLFYYRLWD
LYRKVKRNSTPPLSLFGQLLWREFFYTAATNNPRFDRMEGNPICIQIPWDRNPEALAKW
AEGKTGFPWIDAIMTQLRQEGWIHHLARHAVACFLTRGDLWVSWESGVRVFDELLLDA
DFSVNAGSWMWLSCSAFFQQFFHCYCPVGFGRRTDPSGDYIRRYLPKLKGFPSRYIYEP
WNAPESVQKAAKCIIGVDYPRPIVNHAETSRLNIERMKQIYQQLSRYRGLCLLASVPSCV
EDLSHPVAEPGSSQAGSISNTGPRPLSSGPASPKRKLEAAEEPPGEELSKRARVTVTQMPA
QEPPSKDS (SEQ ID NO:38). The R042 clone was found to be a photolyase receptor based on sequence alignment data. In fact, the R042 clone was found to be the rat paralog of human and mouse clones based on the following observation. The identity between the human and the mouse clones is considerably higher (97%) than between either the human clone and R042 (72%) or the mouse clone and R042 (71%). This lack of a higher identity between the mouse clone and the rat R042 clone is more than that expected from species-to-species differences. Thus, the R042 clone most likely is a different member of the family of photolyase/blue-light receptor homologues. The translational start site was assigned to the second methionine residue from the 5' end based on the alignment data using the human and mouse members of the photolyase/blue-light receptor family.

The R042 clone potentially has two differentially spliced forms at the 3'-end. The difference between these two forms is 142 bp. The shorter form was found in four clones while the longer form was found in one clone.

Northern blot analysis using a sequence from the R042 clone revealed that the expression of the R042 mRNA was strongly upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R053. The primary library screen produced 40 positive signals that were isolated. The following nucleic acid sequence is within the R053 clone: 5'-TTGGCACACAAGTCTGTCTTCAGGACAGCTGATCCATTTTACTTA CRAATTCAGAAAGTAAACATTGGCAGTATGGATCTGGTTACTTCATGGTAACTGCTC TAGAATTTACGCCAAGGCCATCTCTTTTGCCTCACTGTTTAGTGACCGGAGTAAAGC ATGGGGCCACTGAAACTCCACTTTACAATTGGGCTTCTAAATTTAAGGAAAAATTTT TTGATTTAACCACAACTGGATTCCAAAGTTCATCTTATTCYAAATTAGGCCCACTGA GCCTGTGATGTTTTGGAATATATGATTAGTCCACTTGGTTCACTGGATGTTACCTATC ATGTTATGTAGAGAAACAGCCATAACTATTGGTCACGATGTCGTCCTCCGAATTGGG AATGGCTCTGTTGTTGGAAACAAAGTATTTGTAAACACGTTGATCAAAGCGGTGTGC TTTGGCCTTTCCGGGAATCACTGATTATGTTTGAAAACTTCCTTTAATTGTATTTGCA ATAAGCTATTNTCCCTTNTNATGNCNCTGCCATGCTTCCTTGCTTTGCACTGTGGTCG CATGCCATCNGCTGGTTAACCCANGATGGCTTGCTGCNCTGATATNCACCATGCNAA ATACCACTTCT-3' (SEQ ID NO:39).

Northern blot analysis using a sequence from the R053 clone revealed the presence of a 4.9 kb mRNA transcript. In addition, this analysis revealed that the expression of the R053 mRNA was marginally upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R055. The first library screen produced a clone designated R055-7 having a 1.7 kb fragment. A second library screening using the 5'-end of the R055-7 as a probe produced several additional clones having fragments of about 3.0 kb. The following nucleic acid sequence is within the R055 clone: 5'-TGAATTGCAGTAACT AGCCTTGCCTTTCTATTCTGTAGAAATGACAGGGTCTTCACAATCCTTCACCAGTGGC TACTAAGCTATAATTAGCTGAATAGAAAGAATGTGGAAGTGGTCTGAGGCATATAG

AGCATATGCCAAGAACACTACCATATATGGCATCAGCTTTGGTTACCAGAGAAATTT
TCTTAGTCATTAGACCATATAACAGTAATATATCATATGTAAATCTTTAGATTTCAAT
TTGAGAATCCTCCAAAAAAAAAGGAGCAAAGAATGCATAAGCTATGTGTTGGCAAAA
GTAATTTATATTAAAATTTTGACCTGCCTTTGTAAGATTAAGTGGTAAATGTCATAGT
GGTGGGTTTTTACGTCTTAACCAATCTCTGAGGTTTATTTCTCCTGCAGGGGATGGTT
CATGGCCTCTCTTCCCGCTGTAGGAAGATAGCAGAAGGATGAGGATTAATTGTAGCA
TTTCACTGATCCTCGTCCCAGGGACTAGGGACAATAGAAATCTGCAAACATGGAGA
GTCTGTCATAAATATTTGCTTTTTGAAGGTGTTGGTCTTTGTTGATTTCTGTCAGAAA
ATGGCATTATACAAATTATGGGGAGCAACCAACTTTTCTGTTCTGTTTTTGAAGTGCT
ACTATGAACCATTCAGAGTCGTATTTTTTTTTTTTAAAATTTTGGCCAGATATCCCCA
GCTAATGAAAAATAG:TCACCATTCCTTGAAAAAGTTGGAAGCTAGAACCCCCAATT
CCAAATTATTGTTGAAGATGTTTCTCAGGCTACTGTATATAGAAATAATGTTTTTAAG
AAAAATCAAAGAGAGGAGAAAAAAAAAAACCTATGCAGAGACCCTACTACTTTGTGG
TTTCTATTGTCCCTATACATCATTTCAGCAAATCTACTGGCAGTTCTTGTCAGCAAGT
CCTTCAGTGCATATGCTGCACAAAACAAAACAAAAATCTGCATGGCACCAAAAACC
AAACAAGCAAACCAAAAACCCAGACACCCTATGTATCTGTTGGAGGCATGTAGGTG
GTACAAATGACTAGCCATGAGCACACATGGCTTCTTGTCATGTCACTTTTCATAATTA
TTTACTGCAAAATGATTGAGAGGCTTTTGGTGCAGGCAGCCATTAGCCTGCTTCCTTT
GTTACCTCTGGATCACTTTGCAGTAAATTGCAGGTCTTTTAAAAGATTCAAGCTTCGG
TTTTCTCAAAACAAAACAATTATCCTGTCTTACCTGAAAATGCAGGGTTGTGGGCAA
AAGAGGCTGGTTATAATAATGCCCTCATATTGAGTGGTCTGTAAATGGCTGCACACT
TCAGGCACTAGAGTTGCCGAGGATGCGTTGTTAATGTGACCTTGACTGGCTTTACAG
GGGTGTAGAACAGTCTACACGGGCGACTATTTGCATCCATCTTGCTCTCGAGGTGGA
TGGAAATAAGAAAAGGCTGGAGTGTGTAAGTCATGCACATAAGTATTCACTGTAAA
TTTTATTTTCATTTTTAACCCAATTATGGTACTTTGTCCAATGCACAACTGATCTCTCA
GTAGATATTCATTTGAAAATAGTGTGGCCTTGACCAGCGAGAAGGGGAAGAAGTGA
CTTAGCTTGTGTTAAGATGACCTGTTTGCTGAGAGTGGTCATTCTGCAGCACCCTAAT
GTCATGGTTTTGATTAGGGAGAGTTAATGTTTTTGACCCTGAATTGAGTTTTCTTCTA

TTTTTAGGAAGTATCAGAATTGCTCTGATGAGTAACAAAGTTGACTGTTTTGATGTCC
AATCTCAGGTTTTAAAATAGAGTGGTATAAAAGTCCACTGTTACTAATTCTTAAGAC
AATTTTGATTTAGTGTGCCCTAAAAGTCACGTGCATAATAAGGCCTGCTCAGAGGGC
AGGGCCTCCATCTGTTTGCTCCTTTCCATGTTGTACGCACTTCACTTGAAAAGGTGTC
AAGTGACTTTGCATTGTAGATTTCCATTTTAACCCCAACATAGTTCTCAAAGATAAA
GCACTTTTTGAACATGAAATACATGGGTAATGTGTGATGTGGATCATGGTTTCTCAG
GCCCCTAGATAATCCACTTCTGAGTATTGTTCTATGTAAGGAGAATAGAGGTCTTCG
CTAATGTTCGAGTTTGTATTCCTGAATGGAATGCACTTGCTAGTTTCCAATGGATGGG
AGAGTAAACACTGCTGCATTCACAATTGATACGTTGCTTTCCCTTGAGCCTTAAGGT
AACTTTTCTTTTCTGTCAACAACAGCACTGAAGTTCTAGTAAGTGAATGAGATTATCT
GTTTTCAGGGTTGGTTTTAGAGTACTGTAAATTAATTAGCTGTCTTCCTAAAGAGGA
ACTCCCTTTAACTCCCTTCGATAGACTGAAAGTGGGTGTGGGGAGGGGGAGGGAAG
AGAGGGAGGTAGTTTGTAGAAAAAAAAAAAAAAAAAAAAAAAA-3' (SEQ ID
NO:40).

Northern blot analysis using a sequence from the R055 clone revealed the presence of a 7.3 kb mRNA transcript. In addition, this analysis revealed that the expression of the R055 mRNA was marginally upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R061. The following nucleic acid sequence is within the R061 clone: 5'-GGCCCCCCCTANAAGGTCGAGGNTATCGATAAGC
TTNAATATCGAATTCGGCACGAGGCCACCAGGTCTTTGCATTGTCTCTTTAAAAGTG
GTGTATAAGGGGGAAATTGGCAAGACAGACATTTCTAAACAGAGGGGAACACAGAC
AGACAGACAGACAGACAGACACACAAACACACAAACACACACACACACACACACA
CACACACACACACACACACACACACACACACACACACACACACACACCCCAGACTGCGTA
TGTGGCATAACATACAGCTTGCATGGGAAGCAGCCCCCTGCRCATTGCTTATACATC
CTCGAGTCCTTTCATCTTTTTTCCTAAAACGTGTGCACCCGCTATAAAGTGGGTGATG
GGCTCGTCAGAGCTGGGCTGATTCTGTGGCCGGTGACCACCATGCCTCAGGTCCCTC
AACCTCCATCACCCATGGCCCAATCCATAACTGCCACCCTTGAAAACCCAAAGCAGT
CTGAGGGTGCTCTCTGCCTGTCACTCAGAGGCCTGGGACGTTGAACCCAAAAAAGCT

AAACTTATGAAAGCCGGGCTGAAATGGGGCCCGGGGCCTGGGATAGCTCAGGCAGG
GGTTTTCCACTCTGATGTTTCCACTGGGCCAGTTTTGTTTCTTTGTCTCTATTTTCTCT
GTTCATCCCGCTGAGTGTTTGTATCCATGATGATTCCAGCATGAAGTACGTAGCACA
CTCCAGTTAGGAGAAATTTTTTAAAGATACAAGACTAGCGTGGTGGTGAGATGAGAT
AGTCTTCTCGTGCTCGCAGCAACCTGAAGGGGCAATAAGGACAAAGAAGGCCATGT
GGCAGGGTTAGCCCCCTCCAGACCAGGGGTACAACGGACAGTTGTGGTGAGCCTCG
GAAAGGCAGGGGTAACCTTCCCTCTCCGTTCTTCACCCATGGCCAGAGCAAGGCAGG
TAGTGAAAGGGATATGCTTGATGCAGAAAAGCCAGCTCAGGCATGGCAGGTGGGAT
TTATAGCTGGTTTTGTTTAAAGCGAAGGCCTGATATTTGATAAATGCAGTAACCAGC
GGTTGAGAGTGACAAGCCCTTAAATGCGAACATTAATCAAAGGAGAACTTAAACGG
CCCCCTTTACAGAAGGACTT-3' (SEQ ID NO:41).

Northern blot analysis using a sequence from the R061 clone revealed the presence of a 4.9-5.0 kb mRNA transcript. In addition, this analysis revealed that the expression of the R061 mRNA was marginally upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R066. The following nucleic acid sequence is within the R066 clone: 5'-CGAGTTTTTTTTTTTTATGTACTTTGAAAATATAT
TTAAAAACATTAAAAATTCTATATTTAAAACATATATTATATGTTAATTGGTACACTT
AAATAGAACCTGTATTTACAATAGGCTTCTGATGTGGTTAAGTTTTAATGCCAATTTT
TTTTTCAATAACATAATTATATAAATATACTAAAATACAATAAATATTTTTCTTGTTT
TACATGGTGAATAATATCTTTACCATAGAGAGAACAAGGCCACAGACATTTACTTAC
AGTTTCAATGGGAATCACTATAAAAAGCATCAGGCCTGCTGCCATGCATGAAACACT
TCTGCCAAAAAGAGACCACAGCAAGACTTTCAGAACAGAACAGAACAGAACAGGAC
GGAAACAGAACGAACAGAAACAGAGGAGAGATTTTAACAAATCAATCTCAGGTCAA
CATAAACCACCGACATGGAGCTATGATGTATCTTAGTGGGTATGAGAGCCAGCCACT
GACCACACAGTTGCGGAGGGTCTCCTATGAAGCCACCTAATCGACCTGGCCCTTCGA
ATACCGTGAGATTGTGATGGGGCTCCTTTTATTTGTTTGACTAACGTCTCTCAGAATG
AAGCTGCAAAAAGTTAGCATATAGCAGATATTCAAAGCATTCCTTAATAGGTTAAAA
ATGATGACAGAGATTAATGTTGTCAAACGGCACAAAACAATCTAGGCTACGTGAAG

TCTTCCAAAAACAGGGGATTCAGTGGGACTCCAGAAGACAGACTAGTTCTAAAGGA
ACAGTTGAACAAAAAGAAACTATTTGCTGATGGTATCTTCACTCCCTGAGTCACAGT
GGACAGCCACTTTGTTTCACCCTTTCCACTCCTAAGATGAAGCAATTGTTTGCCTCTT
TTTCTGATGCCCAGGAGCCCAGTCAGGTAACCACTAACACATTCGCGCTGGCGGAAA
ACCTCACTAGGGAAATGGGCTTAACACTAGTTCTCATTGGGGCCATTCATTCAGGCT
TCCAGCTTGACTTCTCCTAACCCCAAGAGGTAAAGTGTAGAAGGGACCCTTGTGCTG
AATGGACAGAACTATCAGGAGCTTTCTGTGCTCTTCACTTAAGCAGTATTTCCTCCTG
TGTTCTTGTCTCTTTCACAGTGAAAGCACCTTCCTATGCCTTGTCATTCTAGCCCTTAC
AGACAGACATTGCTCATTCTGCCTAAGTTTTGGTGCTTTTTCTGGTTTTGTTTGTTTGT
TTTCTTCTTTCTTTTTTCCTTTCACCAAAATGTCTCAAAAAAATAAATAAATAAAACC
TAGGCTTCCTGAAGTCTAAGCGCAAAGAAAGTTAAGTCTCTTCACAGCAAACATTTC
CCATCATGCTGCACTGATAGCATCACTGCTATGCCATATTTGGATCCAAAGCTGCTC
CAGGTTAATCCAACTTTATCCATAATTATTTAAAATGGGATGGAGGCCATAAATGGA
TTTGAG-3' (SEQ ID NO:42). This clone is similar to BDNF.

Another IEG nucleic acid clone was designated R089. The first library screen produced a clone having an insert of 0.5 kb. A primary screen with a portion of this clone produced seven positive signals that were isolated. The following nucleic acid sequence is within the R089 clone: 5'-AGTCTGGGACTAAAACGTCACAGCAGAAAAAAAATAAAAAAAAAT
AATTTGCTTTTTCTTTCTTTCATTTAGCAGCATAAATAAGTTTGGCCACTGGGAGTAC
AGTACAGGGGTGGGACAACGATCCCGTATTTGAAGACCTACTTCTAGCACCAGCATC
AAGAACTAAATCCACCTCAGGACTCACAGAACCCAGGACAACTTGCCATCTTTGAGC
AACATATGCATTGAAGAGTGTATATAGAAGCAACAGTAAATAGATTAACAGAGGCT
AATACTGTGATTGATTGACATTGGCAATGGTTGGCAAAAAAAAAAAAAAAAAAAA-
3' (SEQ ID NO:43). A portion of R089 was found to be highly homologous to a region within an EST from GenBank representing a cDNA clone from ae87b04.s1 Stratagene human schizo brain S11 (accession # AA774778).

Northern blot analysis using a sequence from the R089 clone revealed the presence of a 3.8 kb mRNA transcript. In addition, this analysis revealed that the expression of the R089 mRNA was marginally upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R095. The first library screen produced a clone having an insert of 2.0 kb. A primary screen with a portion of this clone produced 53 positive signals that were isolated. The following nucleic acid sequence is within the R095 clone: 5'-ACTTGATAAAATTGTATTTTTTTTTCTACAGTCATTTGTACAATTTG
TTACAAAACCATAGAAGACTACAACTTGTTTTAAATCATTTTTGGTCTGCAAATATGT
AAAATCTGTGGTGCAATTATCATGTATTTACAGGGCCTTGTTAGTCATTTTCAATGAT
TATTTCAACAATGTCACACTCTCAACATAAGACATGGCTTAAGACAAATATATTAGT
ACATANATATTCTGAGAACATATTTCCATNAATGGAAAGTNGCTGCTAATACANATA
CAGAATATACATAAGNTGTTTTCTAGCTTTTTAAAACAGTTTTTAAAATGGNAANGT
GAAAAAAGAGCCCCTAGGANCATTTTATCCCAAAAAAATCCTTACNAAATATTNAA
GGGGCCAGGGGGGGGAATTAAAAATCTAAAAANGGTGGTC-3' (SEQ ID NO:44).

Northern blot analysis using a sequence from the R095 clone revealed the presence of two mRNA transcripts: one 2.5 kb and the other 3.2 kb. In addition, this analysis revealed that the expression of the R095 mRNA was extremely strongly upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R113. The following two nucleic acid sequences are within the R113 clone: 5'-AARGGGRCCACCCCACCGSGCTA
AAGGCCCAGGGGCCCCCCCCTTGGAGMCCCAGGGGTTTTGGCCCMCCCCCTCACCC
AAATGGTCTGCCAATGACCCAGGTACTCACAACATGTTCCAGGAGGAGMCTGGGGC
CAGGATTTTGACCAGAGGGTATGGGAAGGGAAAGGGGAGAAGAAATCGACATTTAT
TTTTATTATTTATTTTAAATGTTTACAWTTTCTTTGTGTTGTTCCAAGCCCTGAATAG
AAACAGATAGCATTAAAGGACTCTGTTCCCACCCCTTCTCTGTCTCTCTCTCCCCCAC
TTGTGCTAACTTAGGATAACACTCTCTATTTCGTTTTGTTTCTAAAGTGATTTGTGGA
CTTGTGCCGTGTGAACTGCATTAAAAAGGTTCTGTTTTCAAAGATCGATTGTCGTTCC
TGTGGGGACAGTGGCTCCTAAGAAATCTGCATTGTAGGAGAAGACAATGAAAGACC

CTGGCCCTGTCTCTCAAAACTTAACTCTCTGTATGATTTAAAAAAAAATTCCATTTAC
TTTACTTTGTGGTTACTTGATTTTGAGGAAGAAAATATTCAACTTTGTATAAAGACTA
GGTATCAGGGTTTCTTTTGCAGTGGGAGTTGTATATATATCGTATTTTGGTATATCGT
AGAAACTCAAGCTTTATGCATCCGTATTTGGGATATGTCAATGACGTGCAGTGAAAT
TTGCTATTAGACCCTGGAGGCAAACGAGTTGTACAAGGTTTTATGGCTCCATGGGGA
ATTCTAATTTCCTTTCTGGGGACCTTTTGTCCCGTTTTTACAGTAATGGTGAAATGGT
CCTAGGAGGGTCTCTCTAGTCGAATTCTCCAGGCAGGACCACGTGCTCAAAAAATCT
TTGTATAGTTTTAAATTTTTGAGGAGTATCTCTGCTCAGAAGCATCTGTGGTGGTGTG
TGTTGCGTTGTTCTGTGTACTGTGTGTGACACAAGCCTACAGTATTTGCACTAAGGA
AAGCTGTTTAGAGCTTGCTGCTATGGAGGGAAGAACATATTAAAACTTATTTTCCCT
CGGGGWTTRTWCWMGTTTTATGTWCTTGTTGTCTTGTTGGCTTTCCTACTTTCCACT
GAGTAGCATTTTGTAGAATAAAATGAATTAAGATCAGMWRWRWRMAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3' (SEQ ID
NO:45) and 5'-AATTCCCCATGGAGCCATAAAACCTTGTACAACTCGTTTGCCTC
CAGGGTCTAATAGCAAATTTCACTGCACGTCATTGACATATCCCAAATACGGATGCA
TAAAGCTTGAGTTTCTACGATATACCAAAATACGATATATATACAACTCCCACTGCA
AAAGAAACCCTGATACCTAGTCTTTATACAAAGTTGAATATTTTCTTCCTCAAAATC
AAGTAACCACAAAGTAAAGTAAATGGAATTTTTTTTAAATCATACAGAGAGTTAAGT
TTTGAGAGACAGGGCCAGGGTCTTTCATTGTCTTCTCCTACAATGCAGATTTCTTAGG
AGCCACTGTCCCCACAGGAACGACAATCGATCTTTGAAAACAGAACCTTTTTAATGC
AGTTCACACGGCACAAGTCCACAAATCACTTTNGAAACAAAACGAAATAGAGAGTG
TTATCCTAAGTNAGCACAAGTGGGGGNGAGNGAGACAGAGAAGGGGTGGGAACAG
AGTCCTTTAATGCNATCTGTTTCTATTCAGGCTTGGAACAACACAAAGAAATGTAAA
CATTTAGNATAAATAATAGAATAAATGTCGGGTTCTTCTCCCCTGTCCCTTCCCATAC
CCNCTGGCAAAATCTGNCCCAGGTCCTCCCGGAACATGGTGNGAGTACCTGGGTCCA
TTGNAGNCCATTTGGNGAGGGCGTGGCCAA-3' (SEQ ID NO:46).

Northern blot analysis using a sequence from the R113 clone revealed that the expression of the R113 mRNA was upregulated in response to the multiple MECS treatment. Specifically,

R113 mRNA expression was induced seven fold by the multiple MECS treatment as determined from Northern blot data using total RNA from rat hippocampus (Table I). In developmental studies, the expression level of R113 was found to be low and unchanged in embryonic as well as post natal development.

Another IEG nucleic acid clone was designated R114. The R114 clone is 3318 bp in length and has a nucleic acid sequence as follows: 5'-GGCACGAGCCGAGGCT
CAGCACAGCACGGATAGGGGCGCGGAGCGCACTGAGAACCCTACTTTCCCGTGAGC
CCGAGCCCGGCAAATGGGCGAATGAAGAAGGAGAGCAGGGACATGGACTGCTATCT
GCGTCGCCTCAAACAGGAGCTGATGTCCATGAAGGAGGTGGGGGATGGCTTACAGG
ATCAGATGAACTGCATGATGGGTGCACTTCAAGAACTGAAGCTCTTACAGGTGCAG
ACAGCATTGGAACAGCTGGAGATCTCTGGAGGCGCGCCCACCTTCAGCTGCCCTAAG
AGCTCACAGGAACAGACCGAGTGCCCTCGCTGGCAGGGTAGTGGAGGGCCTGCTGG
GCTTGCTGCCTGTCCCTCCTCCAGTCAACCATCTTTTGACGGCAGCCCCAAGTTTCCA
TGCCGTAGGAGTATCTGTGGGAAGGAGCTGGCTGTCCTTCCCAAGACCCAGATGCCA
GAGGACCAGAGCTGTACCCAACAAGGGATAGAGTGGGTGGAGCCAGATGACTGGAC
CTCCACGTTGATGTCACGGGGCAGAAATCGGCAGCCTCTGGTGTTGGGAGACAATGT
TTTCGCAGACCTGGTGGGCAACTGGCTAGACTTACCAGAACTGGAAAAGGGCGGGG
AGAGGGGTGAGACTGGGGGATCCGGTGAACCCAAAGGAGAAAAAGGTCAGTCCAG
AGAGCTGGGTCGTAAGTTTGCCCTAACTGCAAACATTTTTAGGAAGTTCTTGCGTAG
TGTGCGGCCTGACCGAGACCGGCTGCTCAAGGAGAAGCCTGGTTGGATGACTCCTAT
GGTTTCTGAGTCACGAGCAGGACGCTCGAAGAAAGTCAAGAAGAGGAGCCTTTCTA
AGGGCTCGGGACGGTTCCCTTTTTCCAGCACAGGAGAGCCCAGACATATTGAAACCC
CGGCCACAAGCAGTCCCAAGGCTTTAGAACCCTCCTGTAGGGGCTTTGACATTAACA
CAGCTGTTTGGGTCTGAATTCGAGAGATGCTCACTGACCTAAAATGCAGACTTGTGA
GGGCCCTGGGGGAGGGTGGGCAGATGGCATGGTCTTCAGGCCAGATGCAAGTTCCC
ATCCTCAGAAAGAAAGCAGAGTTCTTAGTCAGGCCTCAGTAGAACAGTGGAGAGAG
GCTGTCACAGGCCAGGCTGAGCTGAGTCCCTGGAGAGAATGTGTGTATTTGTGTGTG
TGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTATGTGTGTGTGTATGCGTG

TGCATGCACTGTTGTTGTTAGAGGCTGGATGTGACAATAATTGGGAGAGGCAGGAA
AGGAGTCCAGGACAAGCCTATGATATTCCTCCATTACCTTACCCAAGACCTCATTTG
AACATTCTATATGCAAAGGGGCATTTAGCCCTCAGGTTTCCCAGAGGAACTCCCAAT
AAAGACCTGTCTCAGGGACCCCCAACCATTTTTTAATGGTCTGCTTCCCTGACAAGG
CACTGATGCAGGCAAGGGGTTTGTTTTTGTTTTAAGGGTTGGTATCCCAGAATGGAG
CACCGGAAATAGGAAAATCCCTATTTATAGCCCTTCCTAGGACCAAGATTTCACCCA
TGGCTGGGTGCTGGGGACGCAGAACAAGCAGAGGGGTGTGCGTGCGTGCGTGCGTG
CGTGCGTGCATGTGGTGTTGAGGAAGCCTGAGATGCTCCCAGATCTCTAAAGTGCAG
AGGAGAAGCAATGTGCGTTCACCCCGGTGATTCCATAAGCAGCCATCTCTGAGAGC
ACACTCGGCTGCCAGGAGGAAAAACAGGTCAGGCCAATCTCATGGTTATCAATGGA
CCCTAGAGTCATACGCTGCCTGGTCCAGCAGTGAGAGCCCATCCTGACTCCCTGTTG
CCTATCTTAATGCTCCTGCAGGGCAGCAGATGGTTGGGGTGAACCCAGAGATAATAC
CCATACATTGAGAACATTTCTTAGTCTACATCTCATAGTCATTCAGCGAACTGGACA
CATCTACCCGCATCACCCTGGAGGTCAACAGGGGACCCTGAGGGTGGGGCTGATGC
CAGGCACTTTATATAGTGAGCAGGCGTGCAAGTCTGGGACCCAGGGAATCCATCTCA
GCCCCCACCCCTTAGCCAGGAGAGAACAAAGTAGGCCCCTGTTCAAGCCCAGCTCG
GAGGCTGCCTTAGCTCCTCCTTCGCCCCCTCCTGCAGACCCAGCTCAGCTTGATGAG
GTGTGACAACTGCAATTAGAGGCAAGCCGCCTGCTGCCCCCAGAGCATTAAGAGCA
AATTAGAGAAGAAAAATCACAAGAGAAGCTCTTCTGCCTGCAGTCTAGACTCCCAG
GGGACTGGGTGGAGGAAGGAAGAGCTTAGGGCATAGGGATGAGGAGGTAAAAGTA
ACAGCAGGAAGGGTCACCTGCAAGTTCCCACGCAGTTAAATGATAGGTGGCCTTTTT
TTTTTTTTTTTAATCTGTAGCTTTTTGTCAGGCAATGTGCCTATCTCTTTCAGAACAAT
TAATCAGTGGGGTCAAAGGGCCCTGCCATGCTGGCTGCCCCCATCAGGCTACTCAAA
AAGGAAAGCAGTTCCAAGCTCCAGCCTGTGGGCATCAGGCCTATCTGCTCTGGCCTG
GTGTTTATCAGCTAGGCTCGCTCTTTCTGGTCAAATGGGTCCTCATCCATTCTGTCCC
CACTGAACTTCTGTCTCTGGTGAAGGAAGGTAACTGTAGCTGCCTCTGATGGCTGCT
GCAATGTGTGTGGAGAATGAACATGTGAAAACCCCACACCCTGAAGGGTGGCACAT
ATGACACATTTACTCAAGAGGACACAGGACTGGGACGGTGTAGGAAGCCAACTCAT

TTGTTTTGTGGACTAGTCACTGTTCACATTATTTAAATCGACTGACGTGACAGACTCC
TTCTTTGACTGGGCACTGTGACAGAAGGAGAGAACTCAGCAATGGGAAAGCTGGCC
TCCACAGCTACCAAGGCACACAAAGAAATCCAGTTAACCACCACCTGGCCAGAAAA
GGGTCAAGGGACCAAAACAAAATGATTAGCAAGTAATTTTGGCTTCTAAGAGAACC
CACAGGTGTCTGTCACCTTGATCTTTATTTTTCTGCTACACCCAGGAAATGGTTGCTC
ATTTTACCCAGTAGACTCGGAGAAGTTAATGCTTTCAAGGTCACACAGTACAAAGCT
GGGATTGAAACAGTTTGTAACTGACTTCCAATCTTGTGTTCATGCTACCTGGCAAAC
TGTCCATATTTGCTCCACAGCCAGATCCAGAATAACATTTGTCTCCTCTCGTGCAAAA
AAAAAAAAAAAAAAA-3' (SEQ ID NO:47). In addition, the R114 clone contains an ORF from basepair 94 through basepair 993. This ORF encodes a polypeptide of 300 amino acid residues. The translational start site was assigned to the first methionine residue in the ORF. The amino acid sequence of the R114 polypeptide is as follows: MKKESRDMDCYLRRLK
QELMSMKEVGDGLQDQMNCMMGALQELKLLQVQTALEQLEISGGAPTFSCPKSSQEQT
ECPRWQGSGGPAGLAACPSSSQPSFDGSPKFPCRRSICGKELAVLPKTQMPEDQSCTQQG
IEWVEPDDWTSTLMSRGRNRQPLVLGDNVFADLVGNWLDLPELEKGGERGETGGSGEP
KGEKGQSRELGRKFALTANIFRKFLRSVRPDRDRLLKEKPGWMTPMVSESRAGRSKKV
KKRSLSKGSGRFPFSSTGEPRHIETPATSSPKALEPSCRGFDINTAVWV (SEQ ID NO:48).
A portion of R114 from base position 111 to position 210 was found to have 98 percent identity with the mouse G protein-coupled receptor EBI 1 (accession #L31580). This homology, however, ends with position 210. In addition, the 100 bp region of 98 percent identity in the EBI 1 clone appears to be an artifact produced while PCR cloning EBI 1. This "identity" region in R114, however, is not an artifact, since RT-PCR with primers located in the 3' untranslated region of R114 and the middle of the "identity" region (139-164 bp) was used to obtain portions of the R114 clone. In addition, a portion of R114 from base position 143 to 601 was found to have very strong homology with a human EST obtained from prostate tumor (accession # AA595469). This indicates that the entire "identity" region is from one gene and not a product of concatamerization of the R114 clone and EPI 1.

The alignment of the human EST obtained from prostate tumor with R114 revealed a

very high level of identity at the 5' and 3' ends of the overlapping region and a somewhat lower homology in the middle. In addition, 13 base insertions and deletions were identified between the EST sequence and R114. After excluding 7 of the 13 differences because they would have caused a frame shift, the two sequences were translated and compared. This comparison revealed an 81% homology at the nucleic acid level and an 85% homology at the amino acid level. Interestingly, no homology was found between the two sequences before position 143 of R114. Position 143 is six bp before the third methionine residue. Thus, the translational start site of R114 may be the third methionine residue in the ORF.

Further, 95% homology was found to exist at the nucleic acid level (98% at the amino acid level; there is a one base deletion in the EST that is probably an error of sequencing) between the 3' end of the R114 ORF from position 580 to about 987 and the full length of an EST from mouse mammary gland (accession # AA472513).

Northern blot analysis using a sequence from the R114 clone revealed that the expression of the R114 mRNA was moderately upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R198. The following two nucleic acid sequences are within the R198 clone: 5'-TTTKTTTKTAATTTTTTTTTTTTNATTTGGGTTGA
TTCCTTGTNTTTTANTTGCCAAATNTTACCGATCANTGANCAAAGCAAGCACAGCCA
AAATCGGACCTCACCTTAATTCCGTCTTCACACAAAAATAAAAAAACGGCAAACTCA
CCCCCATTTTTAATTTTGTTTTTAATTTTACTTACTTATTTTATTTATTTATTTTTTGGC
AAAAAAATCTCAGGAATGGCCCTGGGCCACCTACTATATTAATCATTTTGATAACAT
GAAAAATGATGGGCTCCTCCTAATGAAAAASCAAGGAAAGGAAAAGGCCAGGGGA
ATGAGCTCAAAATTGATGCCCACKTGGGGAGCATCTGGTGAATAATCGCTCACKTCT
TTCTTCCACAGTACCTTGTTTTGATCATTTCCACAGCACATTTCTCCTCCARAAACSC
GAAAAACACAASCGTKTGGGTTCTGCATTTTTAAGGATAARARARARAAAGAGGTTG
GGTATAGTAGGACAGGTTGTCAGAAGAGATGCTGCTATGGTCACGAGGGGCCGGTT
TCACCTGCTATTGTTGTCGCCTCCTTCAGTTCCACTGCCTTTATGTCCCCTCCTCTCTC
TTGTTTTAGCTGTTACACATACAGTAATACCTGAATATCCAACGGTATAGTTCACAA
GGGGGTAATCAATGTTAAATCTAAAATAGAATTTAAAAAAAAAAAGATTTTGACATA

AAAGAGCCTTGATTTTAAAAAAAAAAGAGAGAGATGTAATTTAAAAAGTTTATTAT
AAATTAAATTCAGCAAAAATTTGCTACAAAGTATAGAGAAGTATAAAATAAAAGTT
ATYHGTTTCAAAMTAVCDTRTCGAMCTCVTCVABCCCGRGGAAKCCMCTASKKCBA
RHSCGGCCCCCACCSCSSYSKAKMTYCATKCTTTTGAWWCCCTTTAGTGAGGGTTAA
NAA-3' (SEQ ID NO:49) and 5'-CAGCCTCTCACTCTCTNGCTCTCTTTCTGTCTCTTCCT
CGCTCCCTCTCTTTCTCTCCTCCCTCTGCCTTCCCAGTGCATAAAGTCTCTGTCGCTCC
CGGAACTTGTTGGCAATGCCTATTTTTCAGCTTTCCCCCGCGTTCTCTAAACTAACTA
TTTAAAGGTCTGCGGTCGCAAATGGTTTGACTAAACGTAGGATGGGACTTAAGTTGA
ACGGCAGATATATTTCACTGATCCTCGCGGTGCAAATAGCTTACCTGGTGCAGGCCG
TGAGAGCAGCAGGCAAGTGCGATGCAGTCTTTAAGGGCTTTTCAGACTGTTTGCTCA
AGCTGGGTGACAGCATGGCCAACTACCCGCAGGGCCTGGACGACAAGACGAACATC
AAGACCGTGTGCACATACTGGGAGGATTTCCACAGCTGCACGGTCACAGCTCTTACG
GATTGCCAGGAAGGGGCGAAAGATATGTGGGATAAACTGAGAAAAGAATCGAAAA
ACCTCAATATCCAAGGCAGCTTATTCGAACTCTGCGGCAGCGGCAACGGGGCGGCG
GGGTCCCTGCTCCCGGCGCTTTCCGTGCTCCTGGTGTCTCTCTCGGCAGCTTTAGCGA
CCTGGCTTTCCTTCTGAGCACGGGGCCGGGTCCCCCCTCCGCTCACCCACCCACACTC
ACTCCATGCTCCCGGAAAATCGAGAGGAAAGAGCCATTCGTTCTCTAAGGACGTTGT
TGATTCTCTGTTGATATTGAAAACACTCATATGGGGATTGTTGGGNAAATCCTGTTTC
TCTC-3' (SEQ ID NO:50). This clone is similar to neuretin (accession # U88958).

Another IEG nucleic acid clone was designated R233. The following nucleic acid sequence is within the R233 clone: 5'-AAACCNAGAACCCCCCTTTGNAGAACCNTTG
TTTCCTTTCAAGCCCAAGGAAGGCGGGGCCCAACCTTTGGTGTTNTTTGAACAGGCC
TTGAACAGGAGGNTWAGGAGAAATTTCCGGTTGTGGAACCCCAACAGGAACCCCTT
GGCACCCCTGGCCCCAAGGTTGTGMAACTTTGGTTTGCTTAATTTGGACCGTTTTTGC
CTTGAGGATTCATGACTTTTTTTTGKGCCCTTGTGAGCCAAGATGTTGGGTTTTCCCA
TCAACAWTAATAACCCCTTGCTTTTTGGGGTGATTCCCCTGGGGAGTTTCCTGATGA
ATTCCCCCACAGCTCCTGGGGTTTTCATCTTGTTCTTACTGTTGTCTGGATTAGGAGG
GCGGAGAGGGTGGACTCCCTGAGACAAGATAAGCAGGTGGAGACATAGAAGAGGG

AGGGACATTTAACATAGTAACATTTTCAGAGGTGACAGAGATGATACACGGGCAGC
TGGAMTTTTGTGAAGGACAGAGGAGCTGGCAGACCCACAGGGCCATACCTTTGAGG
GACAGGTGAATGGCTGGTTACCAGAGACAGGACTGGTAGACAGTCAAGTACCTCAC
TACGATGTGCCAAGAGATYTGGGATCCTGGGAAATGTGTGGAGAAGAGGATTTGAC
ACTCCCCACCCCCAAGGCCCTTCCCCTTTGCTGACAGCATTGCTGTGGTCGTGGCCTG
TTGCCTTGTCCTCTGTCCCTGGGTGGGGCACACCCTCCTGTGCTGTGCTTGCCTTGTG
CATCAATAAACCAC-3' (SEQ ID NO:51). This clone is similar to KIAA0273 (accession # D87463).

Another IEG nucleic acid clone was designated R241. The first library screen produced a clone designated R241-4. This R241-4 clone contained a 2.0 kb fragment and a polyA tail. A second library screen using 5'-end of R241-4 as a probe produced an additional clone designated R241-12. The following nucleic acid sequence is within the R241 clone: 5'-GCANTTTGGAGT
TATTGCTTAAAACCAGGNTAAGGCACTTTGTCCCACAGGACCCAGGAATCNTAAAN
GGGTTGAAATTGGGNCGGGGAACCCCAGGATATAATGCNACTTTTGTTAGGGGGAG
AGTTCAGCTCTAACTGGTAGTAGTGTGAAAGTAAGCACCTTGACTTCAATTTTGGAA
AGCACTTGGTAAATGGAGAGAACTTTGGAGTTTCCCTATCATCTATATCAGTCTTTG
AACACACCCTCAAGTCCCAGCCTCAAGGCTCAATAAAGGACCACATAGCAGGTCTG
AGGCTCACTGCTCTCAGCCCTTAACACAGGGCAGTGGAGAGCAGGGTGATCTTCCCT
CTCTGGAGCTTCTCCTTGGCCTTCTTCTCCACTTGGGCTTCTGCTCAGCAGCAGATAT
ATTCTGGGTTCCATAAGGAATCCAGCTGTCCCAGTGGCTTGACCCTGTCAAGGCAAG
ATATCAACTCTGAGGATGACCCAGTCATGGAGGAAGAGAGTGTGACAAGATCCGCA
GTTTGAAGCAAAACTGTGTTTGGTCTTTTCAAGAAACAAATGGGCACATTGAGTTCT
GTTCAGTGTCAGAGGATATCTTTCCCTTTGCTCCCAGATTTCCAGAAATGGATAATGT
TTTCATTTCTGTGGGAAGGGTCAAGAAACATAAAATTGCTCAACAATGCTTGCTTCC
CTTGAGGGTTGTTGAGCAAAGGCCGATATGCCTCCCTGCATTCTCTTCTACCTCAAG
ATTTTGGAATTCAATTCTGGAACAGAAATTTATTTACACAAGAACACTTGTTGTCAG
CCTTGGTTACTGTGGGAGTTACATAAGGGTGACAGTCTGTATCTTCTAARTTAAACA
GGAACTGGGCTTTGGCGGCCTATTGACCCAGTTTATATCTAAATATAACTGTGGCTC

CAAATGATTGGCCAATAACATTCCCTTTACCTTCAAAGTTTTCTCCATCAGTCATTTC
TGTGGCAGCACAGTTCCAATGTCATATGCCCC:TGCAAATTGTGAAAGTAATTAGTGA
CAAAATAACCCTCCCCCCTTTCAGTGGCCAAACTGTCAGCTGTAGCAGCGCTGCGAA
AGCGAGTACTACACTATGTACGGAAAG:CCTGTTCCTTATCACGGACTAGACTCAAG
AAATGCCATCTCCGAACGGTGGCATTCAAGGTGGTAGTCGTTTGAATGGAACAGTCA
TCTATGTGGACATTGTTAAAGTGTTTTAAAGAGTATTTTGAAAATTAAGTTTACATTT
TACAACTGCTTTATTTTTATTGAAACAATTGTATATAAATATTACCCTCTTTCACTGTT
AATTAAAGTAAACCTAGACCTTGTAGACAAGTGGGTCAACTGATATGTATAGAAGCT
GTGATGTAGACAATACCTTTCTCTTGTGTAAATGGTCATAAATATAGCTGTTCCTGTG
TTTTTATAAGTTGAGGGTATTTTGTTGTTTTATAACAACAAAATTTATTGCATTTGAA
ATGGTTTTTATGTAATAGAATCATGCAAACAGTGAAGGATTATAACATGGTATATGT
AAATGTATAAACTTTAGAAAGAAATAAATACAACAAATTTCAAAAAAAAAAAAAAAA
AAA-3' (SEQ ID NO:52).

Northern blot analysis using the 3'-end the R241 clone as a probe revealed the presence of two mRNA transcripts: one about 7.0-8.0 kb and the other 4.8 kb. In addition, this analysis revealed that the expression of the R241 mRNA was marginally upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R256. The first library screen produced a clone designated R256-8. This R256-8 clone contained a 1.8 kb fragment. A second library screen using 5'-end of R256-8 as a probe produced two additional clone designated R256-2 and R256-3. These additional clones contained each contained a 3.0 kb fragment. The following nucleic acid sequence is within the R256 clone: 5'-GGCACGAGGACAGATTCTGAGA
TGGAAACTTAAATTACATCCCAGAGGCAGGGAAACTATGAAGTCACCGTTCCTAGA
CCACCCCTTACTGAGGTTCCACGGTCACACTGACGGCAGGACCCACAAGGGCAGGG
TATTGGTCTGCCCTCCTTTCTCCTGTCTGTCTGACTTACCTAACTTTGGTCTCGGCTGC
TGACACTTGGAAAGGACCAAATTACTTGATAGTATTTCCCCCTGTTTGTGTAATAGC
CTGAAACCTTGGAGAGGTTCCAGAATACTTCTGTATATAGGGCACAGGTGAAGACAT
TGTCCAAAGCTTATTTATTTATCTATTTATTTACCCTGGCTGAGTAACCACACCAGTA

GGGGGAAAACTAAAATGTGTTGAGTGTAAACAAAGTCACCAGCCTGGCTAGAAATT
CTCCCTGGAAAACATCCATTTTGATACAATGTAAACGTTAGTGTTCACCCTTAGATA
CATGTTGAAAGAGAGCTTTGGTACGCGGAAGTGGCATCTTTGGTCACACACCATGCC
AAAGTGAAGAGGTGGCCAGTGGAGGTCTTCCGGTCCTGTCGGGATCATTTGTGAATA
CATTCTTTGCCCCTCTTAAGTACTTGTTTACTAAACATGTGCAGTGGTAGGTATTAGT
GTTAGATCACAGTGGGCACTTCCCTGGGGATCTGGGGAAGACCAGAGCTTGCAACTC
TGCCTGTTTTGATCCCTATTTCTCACAGTGCTGTATTAAAAAAAATAGGATTTAAGAC
AGATAACCACCTTTACATTGTGAGTGTGTTTGCCTTGTCTAACGACAGATAATTCCTT
AACATTTCTCTTCACCTTAGTACTTTAGGCTAATTATACACGTCTGTCTATGCCATGA
GTAAGTGGACTGTAGTCGGACCAAAAGAAAACAAATGAGCCGTTGGACCATTTGTG
CAGTCAGTTTCTGGTCCTTAGATGTATCCTAAGCAGTAAGTGTCTGATTGTACCCTGG
TGGTATGATCAGTTGTCTCGTAGCTGTCTCAGCTCCACAGTTTACAATGCAAATCTGT
CTCAAGATCTTCACGTCACTGCTGCTGAGAGCAGGGAGAATTCTCTGCAGCTGTTTC
AAAGTTGTGGCCCGGCCTTGAATCCTCTGTTAATTACTGTGTGAGCCAGAGGGAGCT
GCCCAGCAAGGGTGGGCCCCCAGCCGGCAGGGGAACTTTCTAGACTCCCCGCTCATT
CAATTGATCTAGGCATTCGGGCCTGCTACTTGACCATTCTCGCCCTGTGAAATGTCCC
ACACTTTGAAGCAAATACAATTCACAGCACAGTACACACAAAAACCCTGGCATAAG
ACAGGGGAGGTTCTTCTTATTTTGTGAGCCGGTTGCCCTGGAAACGGATAACAAAGG
GCAGCCTTCCACTTCTGGCATAATGGTGGAGCCTCTTTTCTCAGGCTTGACACCTGTC
TGAATAAGAGTGATTAGAGCCGCATAATATCCCTCTCTTGGCTATTGAATATGTGGT
TCACATACCAAACCCTGTAGAAGTTAGAAGACGGTCGTGTTCGTATGTTGTTTGCTT
CCACTACATTTTTGAGGTTTTGTAAAACTGTTATTTTTTTTCACGATGTGAAACTGAA
GGTCAATAAATTATTAGAGATTTTCAAAAAAAAAAAAAAAAAAAAA-3' (SEQ ID
NO:53).

Northern blot analysis using a sequence from the R256 clone as a probe revealed the presence of a 4.0-4.8 kb mRNA transcript. In addition, this analysis revealed that the expression of the R256 mRNA was moderately upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R261. The first library screen produced a clone containing a 1.0 kb fragment with a polyA signal and tail. A second library screen using a portion of this clone as a probe produced 41 positive signals that were isolated. In addition, PCR using T3 or T7 primers along with a R261 sequence specific primer resulted in the 850 bp of additional sequence from a solution containing the phage plug from a first screen. The following nucleic acid sequence is within the R261 clone: 5'-

CTTAAAACCCCTAGATTTCCTGTTA
CATACTAACACAGGTCTTCCCTTTCACTCCAACCCCAGGTTTCAGGCCTCAGAGCCA
TGCTGGGGTTGGAGAAAACTGCATTCCTATGAGGGTAAAAAGTAGCTGCCCTCTCTG
ACCCTTTCTTGCTAGGCTTCATGCGGGATGGGAGAGGGTATCCCCAGGATGGGGACA
GAGGAAGCCTGGCTAGGGCCTTCTAGCCCAATAAGCCAAACAGGAACTATAAGCAG
ATCAAAATCCTACACTAGCTTATTAGGGCCCTGTTAGTTGAAAACCTTGTTGCTGTCC
CAAGTTCTTCAGTTACAACCGAGTACACTTACTCTTCCAACTGTCCTAAGGGTCACTA
CCCAGCCAGCTTTGGATCTTCAGCACTTTTAAAAGCTGAAACTCCCTCTTGCCCTTCT
TGTCTATTCCTCACTGCCAGTTGGGGCCTAGGCTCAGTCCTGGGCAAATGCCCATGA
TCCTGCTGCTGTGGGAAGTTTGATAGGGCATTTGGCTCAAATTTCAAAAGGCCTCGC
TCCTGACCTGATTTCTCGAAGCTCCAGTAGTTCTAGACCCCTCCAATCTCTCATCTGA
CTGGTTGCAAGGCTTATTTTTCTTTTGTACTTTCCTATAGAGCATTTCTGTAGCATTTG
AGTGTGGCGATATTTTTGTTGTGTGTAGATTTCTAAGAACCAACACTACTCAGTCTCC
TGCTAGTCTGACTCCTGAAGCATCAGACCTCGTCATACGGTATTGACTGTGTATGTG
CCTTTCACCTTGAGCATGCTTCAGGATTTTTTTTCTTAAACCACAGAACTTGAATACA
CAAGGGAACCAGAATTCACAAAGTCCTATGCAACCCTAGACAGGAGGAGGTTAGAG
AGTCTGTCTTGATTGGTGATTTCAGAGACCCNAGAGAAATTTGTACCAGTTTGTATT
AATGTCAGTACTACCAGCACTTTGCCAAAACTAAGGATGTCAGAGGGACCTGTTTCT
AGAGTGAGTCCCAATTACATCAAAGGGCAACTTACAGCTTTCTCCAGTAAGTCTGAG
TGGTTCTCTTGAGCTGGTGTCACTTTCTAACCTTTGCCAGTCTAGCCCAGCAGGGCCC
TGTGTGTGTGAGTGCAGTTTGGTGCTGTTTTGGAGTATGCCTGCTCCCCAGCCTGGAA
CCCTCTCAGCAACTTGCTGGGACCTATAATGTCTTAGGTGCAACAAGGACCCTACCA

GAGCTCCTGGGTGGCTTTCAAGATCCACGTAGCTTTGTGTGAGGGGACTGAATGCAG
ACAAACCACAGCCTGCTTCAAATACCTTCTTTCCCTACCACCTAGTTCCAAATGGAA
CCAACAAGTTGAGTGCATCTCTGTTGGGTGTTTTGTGTTGAGACTGGCTGAAGTGAA
AACTCTTTGACTGACCATGTTGTGATGTGTCGACAGACTCAAGGACACAACCACCTC
GAGCTGGTCATGTGGCATGCCTGTGTATGTGTGTAACAGGATTCTGAATGTTAGGTT
GTAATGCTATTCCTGTATGGGAGAAAAAAATAATATAAACAAATAAAAATCTATTTA
AAGCACAAAAAAAAAA-3' (SEQ ID NO:54). Sequence analysis revealed the presence of some homology with EST sequences including that of a cDNA clone from ae69b04.s1 Stratagene schizo brain S11 (accession # AA774320).

Northern blot analysis using a sequence from the R261 clone as a probe revealed the presence of a 4.0 kb mRNA transcript. In addition, this analysis revealed that the expression of the R261 mRNA was marginally upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R272. The first library screen produced a clone that was used in a second library screen. This second library screen produced two additional clones designated R272-1 and R272-2. Clone R272-1 contained a 2.0 kb fragment while clone R272-2 contained a 1.7 kb fragment. The following two nucleic acid sequences are within the R272 clone: 5'-CCATGGGGACTGGTTTGTCACCNATTGCCCATGGNTTGGTT
GGTAGGTGTTTTTTGGTGGACATTTTTGTTTCNCGTTTTGAACTCCAGATTATTGGGT
TTTTGTTTTAATTTATTTTTGTCAGAGGAAAAATAATTTAACATCCATCTCACAGGCT
TGCTTGACTGTTCAGTTCCAAGGTCCTGCTCACTTTTTCTTGTCTTGCCTCTGCTCTGG
CTTTCTTCATGATAGTGCTGGACGTGGAGCTGAGAGTCTCGTTTACTCTAGGCAAAC
CCTCTACCTGAAGCCAGAGCCCAGCACTCCGTACCACCACAGACTTCTGAAGCTGGC
AAAGTTTTAGAAGCTGGGAGTTTTCTGATTCTCTCATTATTAAGTTTCTCCTCAGTCT
TTAGATAGAGGTAAATGTGGGCTTGTAAGAAAAGAAACGAAAGCACGTAATGTACA
CCTATTCTGAATTATGCAAATTAGCTCTTACTCAGGGTCAACTAAATTACTTCAACTC
GCCCTTTAGTTTACTCTTAATTTGCAAAAAGAGAAAAAAGAAGGAAAACTAAATAG
GACTATGATTTGGGGAGCCAAATTGATAATCTGATGTAAAAGTTGCTGTGTTAAACA
TAAATTATTAAGTGTAGACTTTTTTCCTAGGATATTGTATTCATTTTGTGATATCGCC

TAGAATGATGTATTAGATAAAAATCAATTTTGTAAGTATGTAAATATGTCATAAATA
AATACTTTGACTTATTTCTCAAAAAAAAAAAAAAAAAAAAAAA-3' (SEQ ID NO:55) and
5'-GATTTTATATTCAATGTTGTTTATTTAATCCATTGCAGTTGGTGAATGCCTTTT
CCTCCTAGACACCCTGTATTATACCATTTGGGGATTAAGTCAAAGTTAAGTATATTTT
TTTCTTACTTGAGCTCTATATATGCAATTCAGATATCTTCCTGATGACAGTTTTATAT
GTAAATGTAATTTAACTTTCTTTCCGTGTTGACGAAGTTCTGTAGGTGTTAGGGTTAG
AAGTCTCAGCACTCACTTCTCTCACTGGATGTGCAGTGTGCCTGCCATGGCGCACGG
CTTCTCAGTAATGATGCCATCTCTGCTACTTTTACAGAAGGAGAAGTTTACTTTTGAG
GTGGGTATGTGTTGATATCTAAACACTGTGTGTTGCTTGCTTAGATAGGCAAGACAC
ACTGCTGTGCGTGGCTCCTGTGGTGCACCTAGCCCAGGGGAACGTAGCCTCAGTACT
TCCGCTGGCTTCTTCATGCCTAAGAAGCAGGGGCCTTTCTTGTTTGCTGGGCTCTGGC
TTTAAAAGTTGTCCTTTGGGTCTGGAGATGTAGCTCTGTGACAGAACACCAGCTAAT
GTCAGGTCCTGCGGTCAGTCTCTGGTACACACAAGCGCACACTCACATGATGGGGGG
ATGAAAGGCTGTCCTTGTGTAACAGTATTCGATGGGGCGTTGCCTGGATGACGATGT
TTATGTACTCTGAAGGCAGATCCTGAAGGCACCCTGTTCTTCCCTTCCTTGTGTAACT
GAGTCTGCACTAGCTTAGCCACTGTTTTAGAGGCCATCCTAGTGGGCGAACAGGAGG
CATCGCACTGGGTGATGGTTTGCCTTCAGTCCTCAAGTAACAGCGGCCGACTTATGC
CGATGGCTTGTTTGAAATCAAATATTACCAAGTTGGCCTAGTCTGCCTTCTGTGAAG
AAGGGGAGAAAGGAAGGGTGGAAAGGTGGATGGAAAGCCTTTGGGGAACTAGTCT
GATCTCTCAAGGG-3' (SEQ ID NO:56).

Northern blot analysis using a sequence from the R272 clone as a probe revealed the presence of a 1.0 kb mRNA transcript. There appears to be a discrepancy in the length of the R272 mRNA since the Northern blot data indicates a message of 1.0 kb while the cloning data reveals a message length around 2.0 kb. Regardless, the Norther blot data indicated that the R272 mRNA expression level was moderately upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R280. The following nucleic acid sequence is within the R280 clone: 5'-CTTCAGTTCCTTTGAGGGGNCTTTCCTTC

GAAGGGGATACGCCTACCTTTCACGAGTTGCGCAGTTTGTCTGCAAGACTCTATGAG
AAGCAGATAAGCGATAAGTTTGCTCAACATCTTCTCGGGCATAAGTCGGACACCATG
GCATCACAGTATCGTGATGACAGAGGCAGGGAGTGGGACAAAATTGAAATCAAATA
ATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCAACAAATTGATAAGCAATGCT
TTTTTATAATGCCAACTTAGTATAAAAAAGCTGAACGAGAAACGTAAAATGATATAA
ATATCAATATATTAAATTAGATTTTGCATAAAAAACAGACTACATAATACTGTAAAA
CACAACATATGCAGTCACTATGAATCAACTACTTAGATGGTATTAGTGACCTGTAAC
AGAGCATTAGCGCAAGGTGATTTTTGTCTTCTTGCGCTAATTTTTTGTCATCAAACCT
GTCGCACTCCAGAGAAGCACAAAGCCTCGCAATCCAGTGCAAAGCTTGCATGCCTG
CAGGTCGACTCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACC
GCATCAGGCGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCA
CGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGT
CTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAG
CTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTGC
CATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCG
CTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAAC
GCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTGTAATACGA
CTCACTATAGGGCGAATTGGGTACCGGGCCCCCCCTCGAGGTCGACGGTATCGATAA
GCTTGATATCGAATTCGGCACGAGCCGCAGCCGATATGCAGTCCCCGGCGGTGCTCG
TCACCTCCAGGCAAGTTCAGAATGCGCACACGGGYCTCGACCTGACTGTACCACAGC
ACCAGGAGGTGCGGGGTAAGATGATGTCAGGCCATGTGGAGTACCAGATCCTGGTG
GTGACCCGGTTGGCTGTGTTCAAGTCAGCCAAGCACCGGCCCGAGGATGTCGTCCAG
TTCTTGGTCTCCAAAAAATACAGCGAGATCGAGGAGTTTTACCAGAAACTGTACAGT
CGTTACCCAGAAGCCAGCCTGCCCCCACTGCCTAGGAAGGTCCTGTTTGTCGGGGAG
TCTGACATCCGGGAAAGGAGAGCCATGTTTGATGAGATTCTACGCTGTGTCTCCAAG
GATGCCCAGTTGGCGGGCAGCCCAGAGCTGCTAGAATTCTTAGGCACCAGGTCCCCG
GGGGCTACAGGCTTTGCCACCCGAGATCCCTCTGTCTTGGGATGACGACAGCCAGCC
GGCCAGGGGACAGTGATGAGGCTTTTGACTTCTTTGAGCAACAGGGATGAAGTGCA

AGCCACCCACATTGGGCCTGAGCAACAANGAAATGTTGAGAAGGTCCNTGGAAGGA
ANGAGGAGGGAAGGGAGGAAGGANGATAACTTGGGATCCCCCTTGGGGCAATCAAT
GCGGCCTCCCAAAGGAAAGNCCCTAAAG-3' (SEQ ID NO:57).

Northern blot analysis using a sequence from the R280 clone revealed that the expression of the R280 mRNA was upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R286. The first library screen produced a clone that was used as a probe for a second library screening. Briefly, the ^{32}P-labeled probe was used to screen a UniZAP rat hippocampal oligo(dT) primed library (Stratagene). This second screening produced a clone having a 4.7 kb full-length R286 cDNA sequence. The nucleic acid sequence of this rat version of R286 is as follows: 5'-
CTGCCAGCCGAGGCTCCTGCCGC
TGTGACCCGCGCTCCGCCCGCCGCCGGGCCGGGACCCTGATAGCTAATGTCAGAAG
AAAGTGACTCTGTGAGAACCAGCCCCTCTGTGGCCTCACTCTCCGAAAATGAGCTGC
CACCGCCTCCCCCGGAACCTCCCRGCTACGTGTGCTCGCTGACAGAAGACTTGGTCA
CCAAGGCCAGGGAAGAGCTTCAGGAGAAGCCCGAGTGGAGACTCCGGGATGTGCAG
GCCCTTCGAGACATGGTACGGAAGGAGTACCCATACCTGAGTACATCGCTGGATGAT
GCCTTCCTGTTGCGCTTTCTGAGGGCCCGAAAGTTTGATTATGACCGGGCCCTGCAG
CTGCTGGTCAACTACCATGGCTGCAGGCGGAGCTGGCCAGAGGTCTTCAGCAACCTG
AGGCCATCAGCCCTGAAAGACGTTCTTAACTCTGGATTCCTCACAGTGCTGCCCCAC
ACAGACCCCAGGGGCTGCCATGTCCTCTGCATCCGACCAGACAGATGGATACCGAG
CAACTACCCGATCACCGAGAACATCCGCGCCATCTACTTGACGTTAGAAAAACTCAT
TCAGTCCGAGGAGACCCAGGTGAACGGGGTTGTAATCCTCGCCGACTACAAGGGAG
TGAGCTTATCAAAGGCGTCTCACTTTGGCCCCTTTATCGCCAGAAAGGTGATTGGCA
TCCTTCAGGATGGCTTCCCCATTCGGATAAAAGCAGTTCACATAGTAAACGAACCTC
GGATATTTAAGGGCATTTTCGCCATCATAAAACCATTTCTGAAGGAGAAAATTGCAA
ACAGGTTCTTCCTCCATGGGTCTGACCTGAGCTCTCTGCACACGAGCCTTCCAAGGA
ATATCCTCCCCAAAGAGTATGGGGGCACCGCTGGGGAGCTGGACACTGCCAGCTGG
AACGCGGTGCTGCTGGCCTCGGAGGATGATTTTGTGAAAGAGTTCTGCCAGCCTGAG

TCTGGCTGCGATGGTCTCTTGGGCCAGCCCCTGCTGCCTGAGGGGCTGATCTCAGAC
GCGCAGTGTGACGACTCCATGCGAGCCATGAAGTCCCAGCTCTACTCCTGCTATTAG
CCCTCTTCCGGGAGAATCACCATGTGTAATTCCTTCCTTCTTCGAATGCACAGGCTGA
AGATGCCAGGACCTCGGTCTTGCTCCATCACAGTGCAGCACGGAGCTGCCTGCAGAG
ATTTAAGGAGAGCCCATCACAGGCAGACCTCTGACCAGCTAGGTTATTCCAAGAAG
ACATGGAAATTGCCCTGGTGATTCCCAGATGTCTGTACTCTAAGTCTGCAACTGTTA
CTCTGGAAGCTGCATCTGTTTCTTATGCATCTTGGAAAGAACTAGGGTCAAAGTCAC
TCTGAAGTGACCAGGAGTAGACAACTTGATTGATCATGAGTCTGAAACAATTGCCAA
TCCTGAAAGGTGGCCATGCGTGAGACTTTGAGTCTCTTTCCCATAAACTGTAGGTGT
TGACTACTGCTGCTTATCTGCAAAGGTCAGGGTTCAGGCCCCAGTTGGCATTGCTGG
GTCTGGGAAGCACTGCTAACTGAGTGGTAGAAACGCCAGGCCCAGGCAGCACTTAA
AGGTTAAAGGTCAAATTTGGAAGCTAAGGCTATAAATCATCCTGGGTTCCAGGCTTA
AATCTTGCAATGGACACTCTCCCCAAACCATAAAGCCTTAGCTCTGGTTCTCCATGG
AATCATGCAGGTCAACATAAAATACTGGATTCTTGGACTGCGTGGCTAAAAGCACTT
AGACTARGAGTCCAGTGTGTGACTGGATGGATAGGGGCCTCAGCTTGTCAACTCTAA
GTTAGMGMTCCATGGAATGAAGGCCTTGRGGGCTGCTCAAGTTCTGTTAGGTTTCTG
CTTGGAAAGATGACCACCTGGAGGTGGCCGGGCCTTTTTGGTTTGGCTTGGTTTTGT
GTTATAGACACAAGCCTTATGGAAAGGAACCGTCTGGCCTTTAAAGAAATTACTATG
TTCCTGGGAGTTGGTGGTAACCAGCTGCTTTTGCAGATGATGGGTGAACTGGAAAGG
GATGGCTTTTGTGAGGCTGACCAAGTCTTGTACGCGGATGTTGTACAGATTCCTCCC
ACACCGGAGACATTCGTACTATATTAGAAACAGCCACGGACTTGTGCTCTTTCAGTT
TGTGTCCCTGGAAACATACGGGGGGCAGGCTGTTGCTGGTTCACCTGGGGGCCCTGC
CCTCCCAGACACGGGAGTGCTTGTCTAGCGTGGGAGGGCCAGTTGGCCAGATTGTTA
GCTCTGCGTTGGGGTGTCGTAGACAACTGACAGGATTTTAGCCTTAACCCAAGCACT
GAGTGAGGTGATTTTTCCCTTGGCTTTTGGCGTGTCTTTGGTATTCACCATGTATTGT
GGTGTCAGGTAGTGTCAGGTACTGTTGGCTGTGTGTCTCCTAGACTAAGCGGGCGTT
GSATACAGCTTACATACAGTGCTTGGAGACCAAAGGTCAGTTGGTTGTAATAAGCTG
GTCCACCCTTAACAGACTTCCCAAACATYACAGAAGCTYTTATGGMCCTTACCTAAT

AATGCCAATTCTGGAGGACACTCTTTTACCATAGAWKCSAATCCTTGATCTCCTGGC

TCCTGGTTGAGCTTCCGCACTGATACACCCTCTTGRCTGCCCATCAGGGCCATTTGCT

GCTGAGTTCTGCATTGCTTAAKCTSCKGSYGYTTTCTGCCTAAAGGGATGGCCACCC

AGACACCTAAAAAGACCCGGGATGGCTCTCTAGCCTTGGTGGAGAGTCTTATTAGAA

GTTTTCTTTGGGGGATTGGGGATTTGGCTCAGTGGTAGAGCGCTTGCCTGGCAAGCA

CAAGGCCCTGGGTTCGGTCCCCAGCTCTTAAAAAAAAAAAAAGTTTTCTTTGGTAGT

TGGGGAAAAGGCAGAAGGAAAAAAACAAAGGGAAAGATGAATCTCTCAGTCCTAC

CTGGTTCCCTAAATTTAAATCGTGTCATGTGACTAGTTAAGTCTCTTTGACTTAACAA

AGGGACACCAGGTTCTTGGGGAGAAATCTCAGAGCAAAATGTTGCCTGTTGSTAACC

TTCTGGTAACCARAGGARCCTTGATAARCTTARGAGYKGACTGTATGTCCATGCTCT

TGTGACTCTAGAGACTCTGGCACCTCAGGTTNAAGCAGGCTGTGAGCCAGATGTCCT

GGTGCCAAGCAACCCCACTGTTGAGCAGCAGGGGCACCATAGGCCTCAGCTAGGGG

AGCGCACTGGTAGAGCCAGCAAGTGAGCAGGAATCTGACTTTAGGGTAAAAATCTA

GACAGTTCTGACAGCTGGAAGTCAACTTTTCCTCCATTCAAAGTCATGTGGCATTGG

GAAGGGGCTAGGGAAATAGAAGTGGGTTCCAGCTTTATCTTCCTACACAGTCTCGAG

TATAGCATTAACACCGAGTGCTGGACAGAGGTTGTCTGCTGAACACTCAATCCTGCT

CCTGACTGACTCTGGAAATAAGGACATTCCACTCTGCTTGGCGCGGAGATGCCCTAG

TGTGCGGCCGCGGGGGCTTCTCTTTCTCAAGTCCTCTACAGNACTTCCAGGCAGTTC

ATCTTCCTAGGAAAAGGTATGGAGGTTCTGCCTTCATGGTAGAAACACAGGATAAA

ATCTACAGTAAACAACCGGTAAGTGCTGGCTTCTTACGCCTTGGCTTTCTCCAGGCA

CAGGTGGGTTCGACTACTCCCATTTCATCTTTGTAAGCACCTCAGGTTATAGGGCAG

TTTCTTCAGAGTTGGGGGGACTGGAGCCATTCCCCCTGTAATGCCTGAGGTGGCCTT

ACCACCTAGCAGCCAGTTTGGCCAGCAACAGCCACACTGCTGTTATGGTATCATAAT

ACCTCATCCTCGGGTTTCCTTCAGAAAGGRAAAWGCTAACTCAGTTGATGTAAGTGT

TGCTGTGCTGGGATCCTGTCATGTGGGAGGGAACACCAAATACACAGGCTCTCAGG

AGACATCTTGCTAAGGCTTCTCTTTACTGCAGTCTGCTCACGTTGTAAATCTGCCCTC

TGTTCTCCTGACTCARAAAGACTCAGCCMCAAATCAAGAAGCGCCATCAAACGTTCC

TTCTCAKKGGGAACGTGCTCCACAGGAAGGTCCAGWGGGATTTGCARCTAGAGTCA

CGTTTTACTGGKTTGTGAMCAAATTTACTGGTTTTCARTTACCTGGGGKCCTATGKG
KKTTTTMAACCTTTTCCCATMAGGCAGTTAGTAGTAGCCACTTTGGGTTCCTGTGGA
CGTGCCTCAGCTTCTCGGCATAGGAACCCAACAGGTAGAATACTTGAAACTTCTCAG
TGGCCAAGACCTCGATACCCTCTCTGATGGGTGGGAACTGGGCTATTTTCCTGACCA
ATCTAGGCCACCATTTTAGTCCCTGGTCACATTCCTTACTCCAAACTGAAATTCAGTT
TGGCTTTGAGTATGTGCACACGTGGTGGGTTCACCTACTTCAGTGTTGACCAAAAGT
TTATTTTTCTAGTGCATTTTTCTAAATGGTAAAAATATGTAATTTTAGTATGCATGAC
TGGGTCTCCAAAATAAAAACTGAGTGTATTGTGAAAAAAAAAAAAAAAAAAAAAAA
AAAA-3' (SEQ ID NO:58). The following nucleic acid sequence is the ORF for rat R286: 5'-
ATGTCAGAAGAAAGTGACTCTGTGAGAACCAGCCCCTCTGTGGCCTCACTCTC
CGAAAATGAGCTGCCACCGCCTCCCCCGGAACCTCCCGGCTACGTGTGCTCGCTGAC
AGAAGACTTGGTCACCAAGGCCAGGGAAGAGCTTCAGGAGAAGCCCGAGTGGAGAC
TCCGGGATGTGCAGGCCCTTCGAGACATGGTACGGAAGGAGTACCCATACCTGAGT
ACATCGCTGGATGATGCCTTCCTGTTGCGCTTTCTGAGGGCCCGAAAGTTTGATTATG
ACCGGGCCCTGCAGCTGCTGGTCAACTACCATGGCTGCAGGCGGAGCTGGCCAGAG
GTCTTCAGCAACCTGAGGCCATCAGCCCTGAAAGACGTTCTTAACTCTGGATTCCTC
ACAGTGCTGCCCCACACAGACCCCAGGGGCTGCCATGTCCTCTGCATCCGACCAGAC
AGATGGATACCGAGCAACTACCCGATCACCGAGAACATCCGCGCCATCTACTTGAC
GTTAGAAAAACTCATTCAGTCCGAGGAGACCCAGGTGAACGGGGTTGTAATCCTCG
CCGACTACAAGGGAGTGAGCTTATCAAAGGCGTCTCACTTTGGCCCCTTTATCGCCA
GAAAGGTGATTGGCATCCTTCAGGATGGCTTCCCCATTCGGATAAAAGCAGTTCACA
TAGTAAACGAACCTCGGATATTTAAGGGCATTTTCGCCATCATAAAACCATTTCTGA
AGGAGAAAATTGCAAACAGGTTCTTCCTCCATGGGTCTGACCTGAGCTCTCTGCACA
CGAGCCTTCCAAGGAATATCCTCCCCAAAGAGTATGGGGGCACCGCTGGGGAGCTG
GACACTGCCAGCTGGAACGCGGTGCTGCTGGCCTCGGAGGATGATTTTGTGAAAGA
GTTCTGCCAGCCTGAGTCTGGCTGCGATGGTCTCTTGGGCCAGCCCCTGCTGCCTGA
GGGGCTGATCTCAGACGCGCAGTGTGACGACTCCATGCGAGCCATGAAGTCCCAGC
TCTACTCCTGCTATTAG-3' (SEQ ID NO:59). Using the rat R286 cDNA sequence and a

portion of the human R286 nucleic acid sequence, specific primers were designed to amplify the human R286 homologue. After RT-PCR using human hippocampal RNA and the specific primers, the PCR product was subcloned in the TA-cloning vector (InVitrogen) and sequenced with SP6 and T7 primers. The following nucleic acid sequence is the ORF for human R286: 5'-
ATGTCCGAAGAAAGGGACTCTCTGAGAACCAGCCCTTCTGTGGCCTCACTCTCTGAA
AATGAGCTGCCACCACCACCTGAGCCTCCGGGCTATGTGTGCTCACTGACAGAAGAC
CTGGTCACCAAAGCCCGGGAAGAGCTGCAGGAAAAGCCGGAATGGAGACTTCGAGA
TGTGCAGGCCCTTCGTGACATGGTGCGGAAGGAGTACCCCAACCTGAGCACATCCCT
CGACGATGCCTTCCTGCTGCGCTTCCTCCGAGCCCGCAAGTTTGATTACGACCGGGC
CCTGCAGCTCCTCGTCAACTACCACAGCTGTAGAAGAAGCTGGCCCGAAGTCTTCAA
TAACTTGAAGCCATCAGCCTTAAAAGATGTCCTTGCTTCCGGGTTCCTCACCGTGCTG
CCCCACACTGACCCCAGGGGCTGCCATGTCGTCTGCATCCGCCCAGACAGATGGATA
CCAAGCAACTATCCAATTACTGAAAACATCCGAGCCATATACTTGACCTTAGAAAAA
CTCATTCAGTCTGAAGAAACCCAGGTGAATGGAATTGTAATTCTTGCAGACTACAAA
GGAGTGAGTTTATCAAAAGCATCTCACTTTGGCCCTTTTATAGCCAAAAAGGTGATT
GGCATCCTCCAGGATGGTTTCCCCATTCGGATAAAAGCAGTCCATGTGGTGAATGAA
CCTCGAATATTTAAAGGCATTTTTGCCATCATAAAACCATTTCTAAAGGAGAAAATA
GCAAACAGATTCTTCCTCCATGGGTCTGACTTGAACTCTCTCCACACAAACCTTCCA
AGAAGCATCCTCCCCAAGGAGTATGGGGGCACGGCTGGGGAGCTGGACACTGCCAC
CTGGAACGCAGTACTGCTGGCTTCAGAAGACGATTTTGTGAAAGAGTTCTGCCAACC
TGTTCCTGCCTGTGACAGCATCCTGGGCCAGACGCTGCTGCCCGAGGGCCTGACCTC
AGATGCACAGTGTGACGACTCCTTGCGAGCTGTGAAGTCACAGCTGTACTCCTGCTA
CTAG-3' (SEQ ID NO:60). The R286 clones were found to be homologous to a family of transfer proteins for hydrophobic ligands (such as lipid soluble vitamins and phospholipids). Thus, R286 is a lipid transfer polypeptide. The amino acid sequence of the rat R286 polypeptide is as follows: MSEESDSVRTSPSVASLSENELPPPPPEPPXYVCSLTEDLVTKAREEL
QEKPEWRLRDVQALRDMVRKEYPYLSTSLDDAFLLRFLRARKFDYDRALQLLVNYHGC
RRSWPEVFSNLRPSALKDVLNSGFLTVLPHTDPRGCHVLCIRPDRWIPSNYPITENIRAIY

LTLEKLIQSEETQVNGVVILADYKGVSLSKASHFGPFIARKVIGILQDGFPIRIKAVHIVNE PRIFKGIFAIIKPFLKEKIANRFFLHGSDLSSLHTSLPRNILPKEYGGTAGELDTASWNAVL LASEDDFVKEFCQPESGCDGLLGQPLLPEGLISDAQCDDSMRAMKSQLYSCY (SEQ ID NO:61). The amino acid sequence of the human R286 polypeptide is as follows: MSEERDSL RTSPSVASLSENELPPPPEPPGYVCSLTEDLVTKAREELQEKPEWRLRDVQALRDMVRKE YPNLSTSLDDAFLLRFLRARKFDYDRALQLLVNYHSCRRSWPEVFNNLKPSALKDVLAS GFLTVLPHTDPRGCHVVCIRPDRWIPSNYPITENIRAIYLTLEKLIQSEETQVNGIVILADY KGVSLSKASHFGPFIAKKVIGILQDGFPIRIKAVHVVNEPRIFKGIFAIIKPFLKEKIANRFFL HGSDLNSLHTNLPRSILPKEYGGTAGELDTATWNAVLLASEDDFVKEFCQPVPACDSILG QTLLPEGLTSDAQCDDSLRAVKSQLYSCY (SEQ ID NO:62).

Northern blot and *in situ* analysis using a sequence from the R286 clone as a probe revealed the presence R286 mRNA throughout rat brain. For *in situ* hybridization, Dig-labeled cRNA probes were used as described elsewhere (Kuner et al., *Science* 283:5398 (1999)). Specifically, R286 mRNA expression was the highest in the cortex and hippocampus while being moderately high in the cerebellar granule cells, brainstem nuclei, several lateral and medial thalamic nuclei, olfactory bulb, and striatum. In addition, this analysis revealed that the expression of the R286 mRNA was upregulated in response to the multiple MECS treatment. Briefly, a probe from the 3' untranslated region of R286 was used to hybridize a Northern blot containing 2 μg polyA$^+$ RNA from hippocampus from brains of untreated rats as well as rats receiving the multiple MECS treatment. After one day of exposure using the phosphoimager FLA2000 (Fuji), an upregulation of R286 mRNA was detected in the hippocampus (3.72 fold induction) collected four hours after the last MECS treatment. An additional Northern blot analysis using 10 μg total RNA from hippocampus from untreated rats and rats receiving the multiple MECS treatment was performed. In this experiment, the probe was the ORF of R286 and the level of expression was found to be induced 2.4 fold in the MECS treated animals (Table I).

In addition, rats that developed seizures following intraperitoneal injection of kainate or PTZ were analyzed for the expression of R286 mRNA in addition to the mRNA of other IEG

clones (Tables III and V). R286 mRNA expression was observed, by *in situ* hybridization, to be mildly upregulated in the hippocampal pyramidal cell layer, cortex, thalamus, and cerebellar Purkinje cell layer at 6 hours post-kainate injection. At 6 hours post-PZT injection, R286 mRNA expression was observed to be mildly upregulated in these brain structures, while no upregulation was observed at 20 minutes post-PTZ injection or at 1.5 hours post-kainate injection.

Other IEG nucleic acid clones included L073 (concatamer with Krox-20), L125 (oxoglutarate carrier protein), L201 (concatamer), R094 (fra2), and R217 (diacylglycerol kinase; accession #D78588).

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.